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A COMPARATIVE ANALYSIS OF RECOMBINATION AT THE *his 1* LOCUS AMONG FIVE RELATED DIPLOID STRAINS OF *Saccharomyces cerevisiae*

by

(C) Elizabeth A. Savage

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

FALL, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled A comparative analysis of recombination at the *his 1* locus among five related diploid strains of *Saccharomyces cerevisiae*, submitted by Elizabeth A. Savage, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The effect of extra sites of heterozygosity on intragenic recombination was analyzed to establish a clearer understanding of marker interaction. The pattern of intragenic recombination between the *his1-315* and *his1-1* alleles of the *his1* locus in *Saccharomyces cerevisiae* was established by analysis of unselected tetrads and selected tetrads containing one histidine prototrophic spore. Recombination patterns in two three-point crosses, one carrying the temperature sensitive allele *his1-1s*, located distally but proximal to *his1-1*, and the other carrying *his1-30*, located proximally but distal to *his1-315*, were compared to the reference two-point cross. The temperature sensitive alleles were also analyzed in two-point crosses to determine if effects could be attributed to extra sites of heterozygosity *per se*.

The pattern of recombination, consistent for all five diploids, indicated that conversion events were predominately asymmetrical and involved only one chromatid, that proximal polarity existed such that the basic conversion frequencies of proximal markers were higher than the basic conversion frequencies of distal markers in any one cross, and that an excess of exchanges was observed in the distal region. The total pattern was consistent with the distribution of hybrid DNA predicted by the Meselson and Radding model of recombination.

Analysis of reciprocal recombination indicated that the position of the crossover occurred at a variable position within the gene and was associated with conversion. In the context of a hybrid DNA model the most probable explanation of reciprocal recombination was that it was the alternative event to a proximal conversion flanked by a crossover. Since reciprocals occurred with much less frequency than these events, it was

suggested that restoration to the parental form of the chromatid carrying the heteroduplex occurred less frequently than conversion in the other direction.

A strong marker effect was found to be associated with the *his1-30* allele. Crosses which contained this allele showed significantly reduced conversion of other alleles in the cross and also showed a total decrease in event frequency. The suggestion of a conversion restoration disparity such that a correction event was more often to the parental form (restoration) accommodated the data. As might be expected from the preceding explanation of reciprocal recombination, crosses carrying *his1-30* had higher levels of reciprocal recombination.

The marker effect of *his1-1s* resulted in increased co-conversion of the distal and proximal markers and reduced reciprocal recombination within the gene. The explanation for this effect was that the extra site of heterozygosity was preventing the movement of the cross-strand exchange into the gene from a distal point. The suggestion was that the presence of two mismatches in the distal region increased the probability of correction and this could prevent back migration of a cross-strand exchange.

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INTRODUCTION

Intragenic recombination has been shown in many organisms to be primarily the result of gene conversion and not the consequence of reciprocal exchange. The earliest most generally accepted evidence of this was provided by Mitchell's work on *Neurospora crassa* (1955). She showed that the production of wild-type recombinant spores in a heteroallelic cross was the result of a 3:1 segregation of one allele, accompanied by normal 2:2 segregation of the other allele. This departure from normal Mendelian ratios was termed "gene conversion". Mitchell also noted that gene conversion was associated with a high frequency of crossing-over of the markers flanking the gene in which the conversion event was detected. Subsequent studies by other authors indicated that this observation was a consistent feature of gene conversion implying that the two events were directly related (Pritchard 1960; Kitani *et al.* 1962; Case and Giles 1964; Fogel and Hurst 1967). Another aspect of gene conversion which became evident was the existence of a gradient of conversion frequency extending from one end of the gene to the other, a phenomenon described as "polarity" (Lissouba and Rizet 1960; Siddiqui 1962; Lissouba *et al.* 1962; Murray 1963). A study by Olive (1959) provided evidence of another parameter of gene conversion which was to become a very important consideration in model building. Olive detected asci in *Sordaria fimicola* which showed aberrant ratios of spore color markers in heterozygous asci. As well as the 6:2 and 2:6 ratios that define conversion, more unusual ratios of 5:3 and 3:5 were found. These latter ratios indicated that the segregation of the character differences had occurred at the mitosis after meiosis, and the term "post-meiotic segregation" was applied. Post-meiotic segregation,

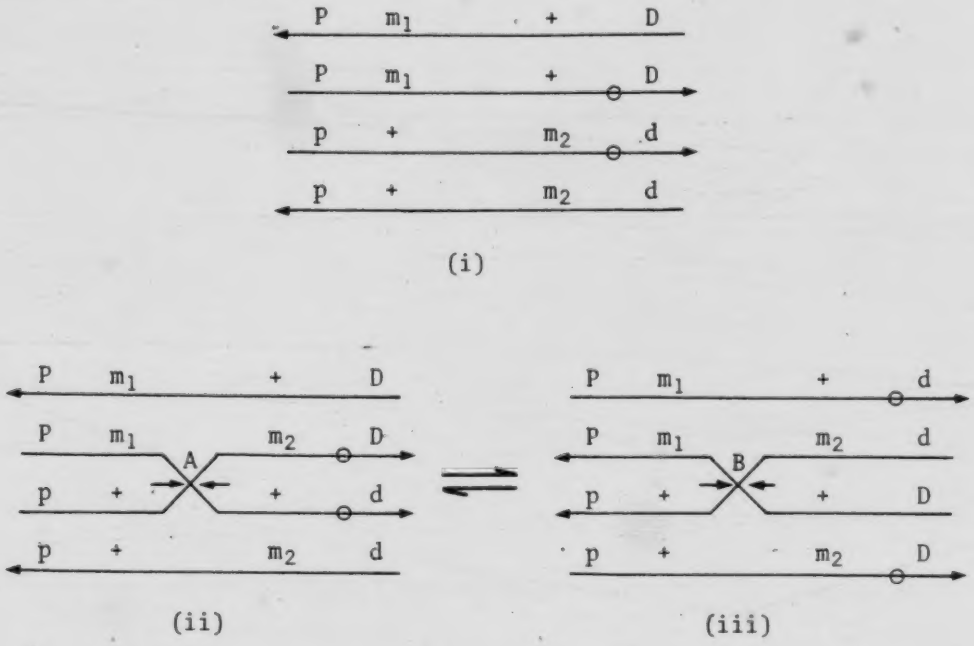
like gene conversion was shown to have a high degree of association with crossing-over of outside markers (Kitani *et al.* 1962; Stadler and Towe 1963).

Models of Intragenic Recombination

Two models of gene conversion were independently proposed to explain the preceding observations: the Whitehouse and Hastings model (1965) and the Holliday model (1962, 1964). Both models share the basic concept that the recombination event involves breakage and rejoining of homologous DNA strands and the formation of a heteroduplex DNA region which is a necessary intermediate step for conversion.

Holliday's model is perhaps the simplest to explain and is illustrated in Figure 1. He postulated that the recombination event begins with the occurrence of breaks in strands of the same polarity in two homologous chromosomes. These breaks are thought to occur at fixed positions outside the gene. After breakage, the strands would unravel from one side of the break and then repair with the other non-sister intact strand. After ligation at the original break points, a "half-chromatid chiasma" joins the two chromosomes and hybrid DNA is generated in the two participating chromatids. If the hybrid DNA covers an area of difference between the two parents a mismatch will result. Correction of the mismatch might then occur, and depending on the direction of correction, restoration or conversion will result. Failure to correct the mismatch would result in post-meiotic segregation. The final resolution of the half-chromatid chiasma can occur in one of two ways: if resolution occurs by scission at A in Figure 1, there will be no recombination of flanking markers. However, if the resolution

FIGURE 1. Diagram of a Holliday-type model of recombination.



m_1 and m_2 are two alleles at one locus

P, D, p, and d are the flanking markers

o indicates site of initial break point

◄ indicates polarity of the strand

(i) The diagram represents two chromatids before recombination.

(ii) After formation of the half-chromatid chiasma. Site of m_2 is in the region of hybrid DNA. Scission at A results in a recombinational event with parental configuration of flanking markers.

(iii) Result of isomerization of (ii). Scission at B results in a recombinant configuration of flanking markers.

occurs after isomerization (by scission at B) the flanking markers will be recombined.

The initial assumption that recombination takes place by breakage and rejoining of chromatids received support from Taylor's (1965) labelling experiments. Molecular support for Holliday's cross-strand exchange was established by Sigal and Alberts (1972), who showed that the half-chromatid chiasma could be assembled with satisfactory bond lengths and angles with no bases unpaired and was free to migrate in either direction by rotation of both duplexes in the same sense. Migration of the cross-strand exchange will result in extension or contraction of the heteroduplex region. It was also suggested that the strands initially involved in the half-chromatid chiasma could be reversibly exchanged with the two outer strands. In one configuration the outside markers are in the parental arrangement and in the other configuration they are recombined.

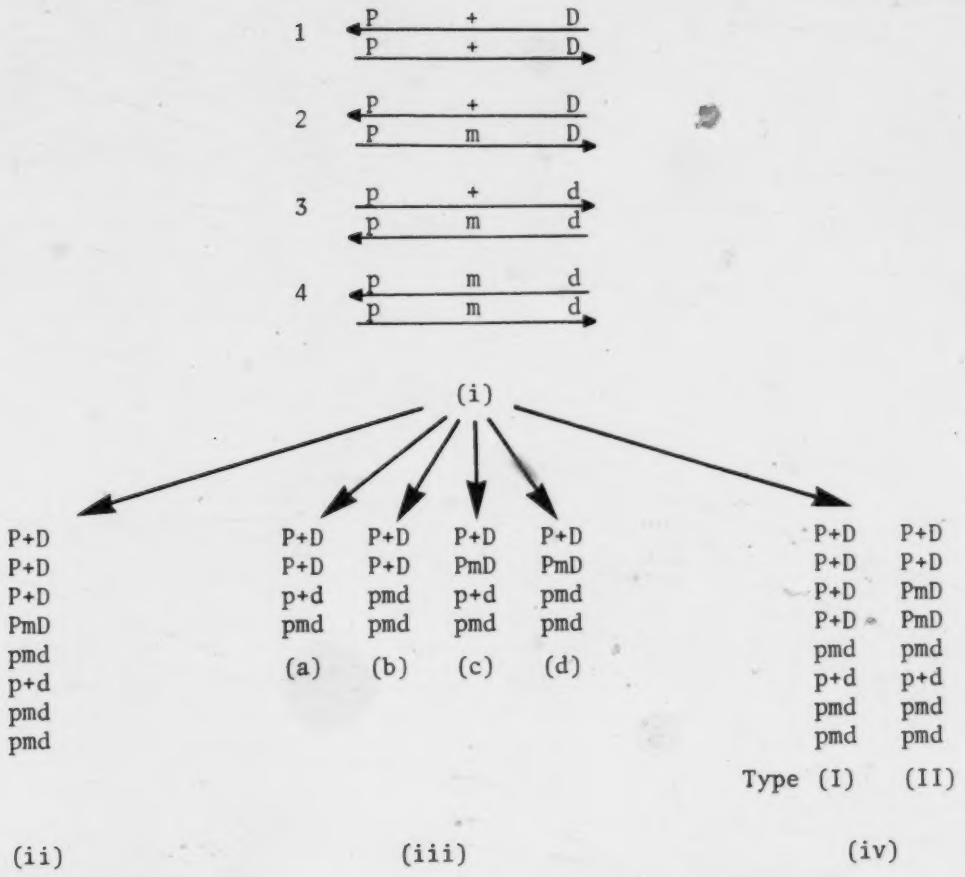
Although no direct evidence of a hybrid DNA intermediate in recombination in eukaryotic systems has been found, there is direct evidence for recombination intermediates which show regions of hybrid DNA in prokaryotic systems (Broker and Lehman 1971; Benbow *et al.* 1974).

Thus Holliday's model, by postulating fixed sites of initiation outside the gene and the establishment of intermediary hybrid DNA, accounts for polarity, gene conversion, and post-meiotic segregation. The strong association of crossing-over with conversion and post-meiotic segregation is a result of two equally possible outcomes of a single event. However, Holliday's model describes a situation which has a symmetrical distribution of heteroduplex DNA region on the two chromatids and one might expect the occurrence of this to be reflected in the data.

The aberrant 4:4 segregation detected by Kitani (1962) supports the notion of symmetrical heteroduplex DNA formation. Reciprocal recombination, too, can be interpreted as the consequence of symmetrical hybrid DNA formation by which the two recombinant spores are the result of correction rather than the result of a cross-over. However, in a number of studies (Lissouba *et al* 1962; Stadler and Towe 1963; Fogel and Hurst 1967), the majority of intragenic recombinant tetrads are asymmetrical and most simply explained as the consequence of hybrid DNA formation on one chromatid.

The problem of symmetrical versus asymmetrical distribution of hybrid DNA has been reviewed by Catcheside (1977). Figure 2 illustrates symmetrical distribution and shows the tetrads which can result depending upon correction parameters. Fogel and Mortimer (1974) suggest that if independent correction of mismatches can occur equally and in either direction (+ → m, m → +) on each heteroduplex, then the frequencies of ascus classes a, b, c, and d are equal as shown in Figure 2. If conversion is accompanied one-half the time by exchanges of outside markers, as seen in the data of Hurst *et al*. (1972), then it would be assumed that the frequency of two-strand double exchanges should be about one-quarter the total detected conversion frequency. However, Fogel and Mortimer's data (1974) on recombination at the *arg4* locus in *Saccharomyces cerevisiae* show that this is not the case. They observed 14 two-strand double exchanges and predicted 80. Furthermore, these 14 exchanges could be assumed to be the result of independent double exchanges since they also observed nine three-strand double and six four-strand double exchanges in the same interval. Their data are not incompatible with a symmetrical hybrid DNA formation in which correction

FIGURE 2. Conversion tetrads (or octads) predicted from symmetrical heteroduplex DNA formation. (Only asci with parental flanking markers (PD, pd) are included.)



(i) Diagram illustrates the formation of symmetrical heteroduplex DNA before resolution.

(ii) No correction. Aberrant 4:4 segregation as detected by Kitani (1962)

(iii) Correction on both chromatids as described by Fogel and Hurst (1974).
- (a) conversion tetrad (3:1)
- (b) non-recombinant-like tetrad
- (c) two-strand double recombinant tetrad
- (d) conversion tetrad (1:3)

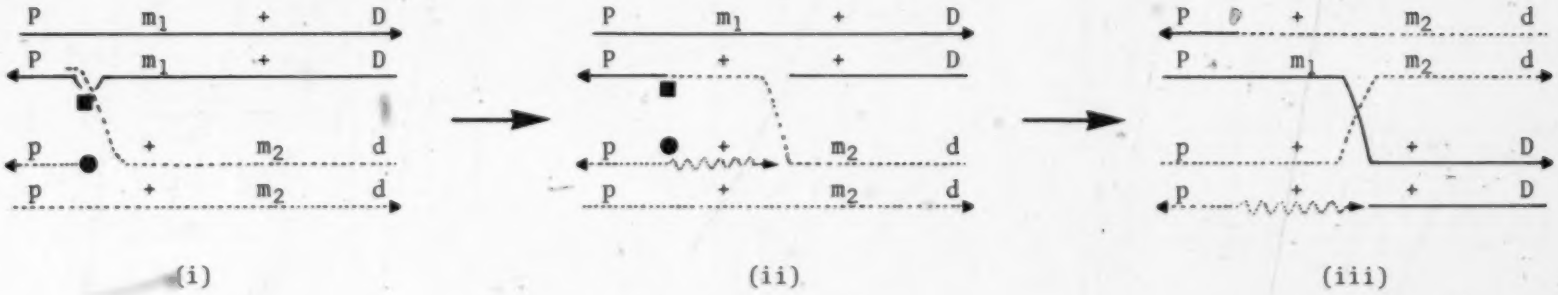
(iv) Correction on one chromatid as described by Stadler and Towe (1967). Only correction of chromatid 2 is shown.

predominantly involves strands of opposite polarity. As can be seen in Figure 2, this would result only in the appearance of classes a and d.

Stadler and Towe (1971) did a similar analysis of 3:5 and 5:3 segregations at the *w17* locus in *Ascobolus*. These segregations are assumed to occur when one chromatid remains uncorrected at the conclusion of meiosis. A 5:3 (or 3:5) segregation could have two possible distributions of outside markers depending on which mismatch was left unrepaired and assuming that the mismatch repair of the remaining chromatid could be repaired to either allele. This is illustrated in Figure 2. A symmetrical distribution of hybrid DNA would result in the same frequency of type I and type II tetrads. In an asymmetrical hybrid DNA no correction would be involved and only type I tetrads will result. The latter distribution is indicated by Stadler and Towe's results.

The conclusion reached by these two studies is that the data are most easily explained by an asymmetrical distribution of hybrid DNA although symmetrical distribution must exist. Several authors (Holliday 1974; Whitehouse and Hastings 1965; Paszewski 1970; Stadler and Towe 1971; Sobel 1972; Catcheside and Angel 1974; Catcheside 1977) have proposed models to account for an asymmetrical and symmetrical distribution of heteroduplex DNA. The model proposed by Meselson and Radding (1975) is illustrated in Figure 3. Their model postulates a single break followed by displacement of the broken strand by a DNA polymerase. This strand can then pair with its complementary homologous strand with the effect of causing a second strand displacement. It is then suggested that this newly displaced strand is nicked and eroded by an exonuclease which is coupled to the enzyme responsible for the initial

FIGURE 3. Diagram of a Meselson- and Radding-type model of recombination. The configuration before recombination is the same as in Figure 3(i).



(i) A single-strand break, ●, in one chromatid is followed by displacement of the cut strand and its re-pairing with its complementary sequence in the homologous chromatid. This induces a single site break, ■, in the second chromatid.

(ii) DNA polymerase activity at the first break, ●, coupled with exonucleolytic activity at the second break, ■, results in further strand transfer and the formation of asymmetrical heteroduplex DNA.

(iii) Dissociation of the enzyme complex allows the structure to isomerize to a Holliday-type figure. The flanking markers are recombined. Further isomerization can yield a structure with the flanking markers in the parental configuration.

polymerase activity. The strand transfer proceeds in a polarized direction generating asymmetrical hybrid DNA. It is thought that disassociation of the polymerase complex allows for the configuration to undergo isomerization as shown in Figure 3. The isomerization results in a half-chromatid chiasma which is recombinant for outside markers, and by further migration is capable of generating symmetrical hybrid DNA. In support of their model, Meselson and Radding cite molecular evidence for strand displacement, strand assimilation, branch migration, and mismatch repair. Genetic evidence also lends support to the Meselson and Radding model. Asymmetry of initiation of recombination is suggested by Angel *et al.* (1970) in their work on the control of recombination in *Neurospora crassa*. They describe a locus, *cog*, which appears to be involved in the initiation of recombination at the closely linked *his3* locus. The active form of the locus, cog^+, promotes recombination between heteroalleles at *his3* and the increase in cog^+ x *cog* crosses occurs preferentially by conversion of the *his3* allele on the cog^+ chromatid. Further analysis of this locus (Catcheside and Angel 1974) in a translocation heterozygote in which the break point occurred within the *his3* locus suggests that the recombination events initiated at cog^+ are unable to cross the discontinuity in *his3* to involve the *his3* alleles past the break point in a recombinational event. If the cog^+ initiation is on the wild-type homologue, recombination events can pass the interchange point to involve *his3* alleles past this point.

Although the existence of an asymmetrical initiation site suggested by the work on the *cog* locus supports the Meselson and Radding model, the prevalence of conversion on the strand carrying the initiation site

is not compatible with the model. Additional assumptions as to the nature of initiation or of this particular site are necessary. For a discussion of one such modification, see Catcheside (1977).

Further genetic support of the Meselson and Radding model comes from Paquette and Rossignol (1978) who examined the conversion spectra of 15 mutants in the *b2* locus of *Ascobolus immersus*. A polarity is present at this locus with mutants in the left portion having a higher total conversion frequency. The frequency of aberrant 4:4 segregation is higher for mutants in the right end. This is interpreted to mean that the left-hand region shows a higher frequency of hybrid DNA formation and that the relative frequency of symmetrical hybrid DNA increases from left to right. This would be compatible with the hybrid DNA formation predicted by a Meselson and Radding model, that is, asymmetrical at the high conversion end and symmetrical at the low conversion end.

The preceding discussion has centered on recombination models which favor correction of mismatches in heteroduplex DNA as the basis for gene conversion. Alternative models are available (Stahl 1969; Pasjewski 1970; Boon and Zinder 1969) in which non-Mendelian ratios are obtained by extra DNA synthesis so that one or both nucleotide chains in one or both chromatids are duplicated. Replacement of a section of one of the chromatids by one of the copies of the corresponding region from the other chromatid results in gene conversion. The models of Stahl and Boon and Zinder account for post-meiotic segregation by postulating short regions of hybrid DNA at the sites of rejoining. Long stretches of co-post-meiotic segregation would not be expected. However, Pasjewski's model can accomodate these observations. Certain allele specific

characteristics of gene conversion and marker effect interactions, which will be discussed later, cannot be readily reconciled with any of these models and are better explained by postulating that the heterozygosity in heteroduplex DNA induces a repair process.

Reciprocal Recombination

Although gene conversion accounts for the majority of intragenic recombination, tetrads are recovered which contain two recombinant products and show 2:2 segregation for all the markers. These tetrads represent reciprocal recombination and the most obvious explanation for these events is to postulate a cross-over between the intragenic markers.

The exact nature of reciprocal recombination within the gene, however, is open to debate. In Fogel and Hurst's study (1967) certain aspects were observed. Their study included three different crosses involving different alleles at *his1*. The frequency of proximal and distal allele conversion was relatively consistent for all crosses, however, the frequency of reciprocal recombination was different in each cross. The variation in the frequency of reciprocal recombination was not strictly correlated with the distance between the markers. The diploid carrying the two outermost markers did not have the highest frequency of reciprocal recombination. In addition, it was found that reciprocal recombinants were predominantly recombinant for outside markers, whereas non-reciprocal recombinants carried the parental configuration of outside markers in approximately half of the cases.

Several explanations for reciprocal recombination within the gene have been suggested. Kushev (1974) favors the idea that they arise from allele conversion on two chromatids; Catcheside (1977) offers the

suggestion that the reciprocal recombinants in Fogel and Hurst's data arose from events originating within the *his1* gene, that is, an event different from the conversion event which is most likely to have originated outside the gene; finally, it is possible that reciprocal recombinants result from hybrid DNA covering one site or the other with the associated exchange occurring between the two alleles. The correction of the mismatched hybrid DNA in these cases must be to the parental strand.

Marker Effects

The preceding hybrid DNA models establish the basis for a recombination event by suggesting the distribution of hybrid DNA. However the genetic outcome of such events is dependent upon what happens to a mismatch in the hybrid DNA and conditions leading to the correction of the heteroduplex remain enigmatic. In some instances changes in recombination pararmeters have been shown to be dependent upon the presence or absence of certain markers. These changes are termed "marker effects" and have been reviewed by Stadler (1973), Catcheside (1974), and Hastings (1975).

It has long been observed that mutants apparently mapping close together give different rates of recombination with another site (Kruszewska and Gajewski 1967; Gutz 1971). Moore and Sherman (1975) showed conclusively in *Saccharomyces cerevisiae* that rates of recombination between *cyc1* mutants of known altered nucleotide sequences did not necessarily correspond to the physical distances separating the mutant sites. Further work (Moore and Sherman 1977) showed that even mutants in the same base pair of the same codon (the initiation codon

of the *cyc1* gene) gave different rates of recombination when crossed to the same second site, most probably indicating that different mutants have different conversion properties.

Leblon (1972) has studied several mutants in gene *75* of *Ascobolus immersus* and has established that in addition to different basic conversion rates, different mutants have different conversion spectra. The conversion spectrum has two components; first, the ratio of conversion to post-meiotic segregation, and second, the direction of conversion, whether preferentially from mutant to wild type or wild type to mutant. The ratio between the frequency of conversion to mutant and frequency of conversion to wild type was defined as the "dissymmetry coefficient". Leblon showed that there was a correlation between the conversion spectrum and the mutagenic origin of the mutant, suggesting that the spectrum is dependent on the nature of the heterozygosity.

Hastings (1975) suggested that marker effects may not be classified solely on the basis of Leblon's (1972) conversion spectra. Hastings describes an additional marker effect concerned with the frequency of use of the opportunity to convert. Historically, the term "opportunity to convert" was introduced by Rossignol (1969) to explain his data on gene *75* in *Ascobolus immersus*. Mutants at this locus can be classified according to their "dissymmetry coefficients". Within each class alleles showed polarity of conversion rates (low at the left end of the gene to high at the right end). The slope of the gradient varied for different classes and since mutants belonging to different classes were scattered with respect to position within the gene, the overall polarity of the gene was obscured. Rossignol suggested that mutants belonging to different classes showed different frequencies in their use of the

opportunity to convert. It can be predicted that if the opportunity to convert was not taken, then mutants with low conversion frequencies in regions of high hybrid formation should show high frequencies of post-meiotic segregation. Hastings (1975) suggested that a marker effect exists which affects the opportunity to convert by modifying the prevalence of free-conversion-heteroduplex DNA. Free-conversion-heteroduplex DNA may be thought of as heteroduplex DNA which is eligible for free correction. He favors the idea that there is a precursor to free-conversion-heteroduplex DNA and suggests the possibility that it is a heteroduplex DNA which either may become free-conversion-heteroduplex or is restored back to the parental form of the chromatid on which it occurs. The marker effect which acts on the opportunity to convert could do so by affecting this decision.

Marker Effect Interactions

It has been observed that one heterozygous site can influence conversion at another heterozygous site. Leblon and Rossignol (1973) showed that in *Ascobolus immersus*, when a mutant allele with a high frequency of post-meiotic segregation in a one-point cross was put in a two-point cross in coupling with an allele showing low post-meiotic segregation, the first allele was co-converted with the second allele and its frequency of post-meiotic segregation was greatly reduced. It seemed that correction induced at one site of mispairing could travel along the chromatid to involve a second mispaired site. Additional data in this study showed that, although there was a polarity of heteroduplex formation, the correction of mismatches was not polarized. The epistasis of gene conversion to post-meiotic segregation shown by this study

provides further support to the notion of a heteroduplex region of DNA as an intermediate in recombination.

Stadler and Kariya (1969) studied the effect of including an additional site of heterozygosity at the *mtr* locus in *Neurospora crassa*. They showed that the presence of a temperature sensitive allele (silent marker) increased the conversion frequency for markers in all parts of the locus and reduced recombination frequencies between certain alleles. They suggested that the presence of the additional site of heterozygosity increased co-conversion of the other markers, that is, a long segment was co-converted with the temperature sensitive allele. The increased conversion frequencies for the other sites suggested that heteroduplex DNA was being increased at these sites.

Increased co-conversion as a response to extra sites of heterozygosity has been observed in *Saccharomyces cerevisiae* (Hurst *et al.* 1972). However, this was not accompanied by increased conversion frequencies of the alleles involved. Fink (1974) described a situation in yeast where the presence of a heterozygous site, a deletion *his4c-290*, caused a significant reduction in the conversion of another heterozygous site. The conversion frequency of that second site returned to normal when the deletion was homozygous. Fink suggested that the depression of the conversion frequency of the second site was a result of aberrant pairing in the deletion heterozygote.

Present Study

It is increasingly apparent that allele specific effects do influence intragenic recombination in eukaryotic systems. Often, however, the studies which reveal these effects involve experimental systems which are subject to partial analysis. Not all events may be detected (Goldman 1974); identical alleles for a comparative analysis may not be available (Stadler and Kariya 1969); or, in some instances, use of spore color markers may cause some ambiguity about genotypes (for discussion see Fogel *et al.* 1971).

The present study was designed to produce a thorough analysis of intragenic recombination in *Saccharomyces cerevisiae* in order to gain a more complete understanding of the interaction of heteroalleles in the conversion process. The *his1* locus, which codes for N-1-(5'phospho-ribosyl) adenosine triphosphate: pyrophosphate phosphoribosyl transferase (E.C.2.4.2c) (Fink 1964, 1965), was chosen for examination. Previous studies, using selected tetrads at this locus (Fogel and Hurst 1967), established certain techniques for allele identification and provided a reference baseline. Furthermore, several mutants, in particular temperature sensitive mutants, are available at this locus (Korch and Snow 1973).

The overall plan of this study was to analyze the products of meiosis from related diploid strains in order to compare patterns of conversion in the presence and absence of extra sites of heterozygosity. A two-point cross, with a proximal allele, *his1-315* and a more distal allele *his1-1*, was used as the standard reference cross. Two three-point crosses involving these markers and additional temperature sensitive markers were compared with the standard. In one cross the

temperature sensitive marker was *his1-1s*, an allele which is proximal but close to *his1-1*; in the other cross *his1-30* provided the additional heterozygosity. This allele was at first thought to be proximal to *his1-315* (Korch and Snow 1973) but this study indicated that this allele is distal to but near *his1-315*. Two-point crosses involving the temperature sensitive alleles with either the proximal or distal sites were also analyzed to determine if a marker effect detected in the three-point cross was the result of the extra heterozygosity *per se*, or if it was a marker specific effect. Figure 4 illustrates the arrangement of the alleles of the five strains used in this study.

Since the additional sites of heterozygosity were temperature sensitive, these markers could be treated as "silent" markers in selected tetrad dissections. These were done to increase the number of recombinant events available for analysis, and in particular to look at reciprocal recombination within the gene.

The analysis of reciprocal recombination in the presence and absence of additional sites of heterozygosity should help determine whether the event is region specific; whether conversion is associated with reciprocal recombination; and whether the event is subject to marker effects in the same way as that of conversion events. The outcome of this type of analysis may be useful in understanding the nature of reciprocal recombination more fully.

FIGURE 4. Configuration of the *his1* alleles in the five diploid strains analyzed.

Z2367	Standard cross	$\frac{315 \quad +}{+ \quad 1}$
LZ6	Three-point cross with the additional site of heterozygosity in the distal region	$\frac{315 \quad 1s \quad +}{+ \quad + \quad 1}$
LZ11	Three-point cross with the additional site of heterozygosity in the proximal region	$\frac{315 \quad + \quad +}{+ \quad 30 \quad 1}$
LZ4	Two-point cross with the temperature sensitive allele *his1-1s*	$\frac{315 \quad +}{+ \quad 1s}$
LZ10	Two-point cross with the temperature sensitive allele *his1-30*	$\frac{30 \quad +}{+ \quad 1}$

MATERIALS AND METHODS

Media

YEPD (Yeast Extract, Peptone, Dextrose)

1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose and 2% Bacto-agar in distilled water.

MC (Mortimer Complete)

0.67% Bacto-yeast nitrogen base without amino acids, 2% glucose, 2% Bacto-agar in distilled water, and 100 ml of stock solution per litre of medium. Stock solution contains 20 mg each of adenine, arginine, histidine, lysine, methionine, tryptophan and uracil, 30 mg of leucine and 350 mg of threonine in a total of 100 ml.

Omission Media

MC lacking one or more of the components of the stock solution and referred in the text as "supplement"$^{-}$.

Low Histidine, Adenine^{-} Medium

Omission medium lacking adenine and with reduced histidine (2 mg/litre). Used to score mitotic or meiotic recombinants in allele identification tests.

Sporulation Medium (F^{+})

1% potassium acetate, 0.1% glucose, 0.25% yeast extract, 2% Bacto-agar in distilled water and supplemented as for MC. Used to induce sporulation.

Strains

Diploid Strains

Six diploid strains were used in the study and their genotypes are given in Table 1. Z2367 containing *his1-1* and *his1-315* was obtained from S. Fogel, Brooklyn College, New York. The other five diploids

TABLE 1. Genotypes of diploid strains used in this study.

Diploid Strain	Genotype								
Z2367	α	+	+	*his1-315*	*arg6*	*trp2*	+	*le1*	*ade1*
	a	*ura3*	*hom3*	*his1-1*	+	+	*ade6*	+	*ade1*
LZ4	a	+	+	*his1-315*	*arg6*	*trp2*	*ade6*	*le1*	*ade1*
	α	*ura3*	*hom3*	*his1-1s*	+	+	+	+	*ade1*
LZ10	a	+	+	*his1-30*	*arg6*	*trp2*	*ade6*	+	*ade1*
	α	*ura3*	*hom3*	*his1-1*	+	+	+	*le1*	*ade1*
LZ6	a	+	+	*his1-315,1s*	*arg6*	*trp2*	*ade6*	*le1*	*ade1*
	α	*ura3*	*hom3*	*his1-1*	+	+	+	+	*ade1*
LZ11	a	+	+	*his1-315*	*arg6*	*trp2*	+	*le1*	*ade1*
	α	*ura3*	*hom3*	*his1-30,1*	+	+	*ade6*	+	*ade1*
LZ13	a	+	*hom3*	*his1-315*	+	+	+	+	*ade1*
	α	+	+	*his1-1*	*arg6*	*trp2*	+	+	*ade1*

LZ4, LZ6, LZ10, LZ11 and LZ13 were constructed from this diploid. LZ13 is a control diploid and carries the same histidine alleles as Z2367 with the flanking outside markers, *hom3* and *arg6*, in the alternative arrangement. The remaining four diploids carry different *his1* alleles. The additional *his1* alleles were obtained from R. Snow, University of California, Davis, California. Strain JB13 was the source of *his1-1s* allele and strain 66A4-143 was the source of *his1-30* allele. The construction of these diploids is presented in Figure 5 which also included the genotypes of the haploids involved.

The mutagenic origin of the *his1* mutants used in this study is as follows: Alleles *his1-315*, *his1-1s* and *his1-1* were isolated after U.V. irradiation and *his1-30* was isolated after ethyl methanesulphonate treatment.

Haploid Tester Strains

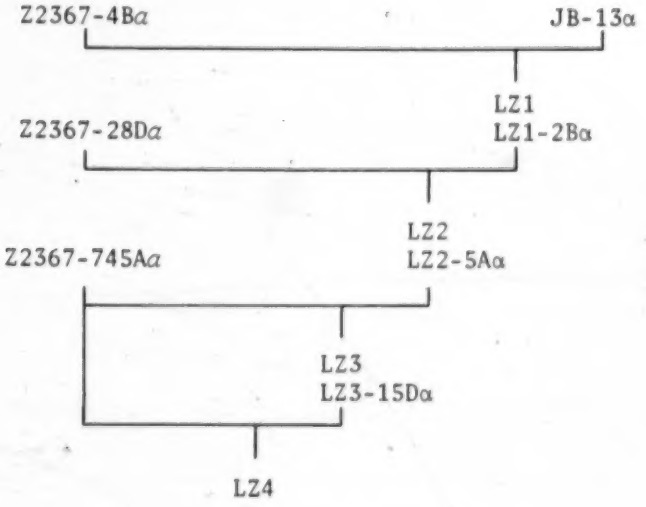
Eight haploid strains, each of which carries a single specific *his1* mutant allele, the *ade2-1* mutant allele, $ade1^+$ allele and one other mating type allele, were constructed for allele identification tests. These strains are referred to as T315a, T315α, T1a, T1α, T1sa, T1sα and T30a, T30α. The number in each denotes the *his1* allele they are carrying.

Sporulation and Digestion

Diploids were grown in YEPD medium for 48 hours at 30°C and then transferred to F^+ medium. They were then incubated at 26°C for six days to obtain good ascus formation. Prior to dissection, asci were transferred to 0.5 ml of a 1:10 dilution of glusulase (Endo Laboratories) in distilled water and incubated for 3-5 minutes at 37°C. The digestion solution was then further diluted with 5 to 10 ml of distilled water,

FIGURE 5. Construction of diploid strains used in this study.

(a) Pedigree of LZ4

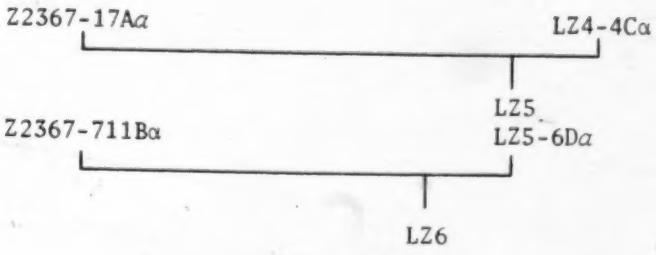


Genotypes of haploid strains:

Z2367-4B	*a*	*ura3 hom3 arg6 trp2 ade1*
Z2367-28D	*a*	*hom3 arg6 trp2 le1 ade1*
Z2367-745A	*a*	*his1-315 arg6 trp2 le1 ade6 ade1*
JB-13	α	*his1-1s ade2-1*
LZ1-2B	α	*ura3 his1-1s ade1*
LZ2-5A	α	*ura3 hom3 his1-1s ade1*
LZ3-15D	α	*ura3 hom3 his1-1s ade1*

FIGURE 5 cont'd.

(b) Pedigree of LZ6



Genotypes of haploid strains:

Z2367-17A	α	*arg6 ade1 ade6*
Z2367-711B	α	*ura3 hom3 his1-1 ade1*
LZ4-4C	*a*	*his1-315,1s trp2 ade1 ade6*
LZ5-6D	*a*	*his1-315,1s arg6 trp2 le1 ade1 ade6*

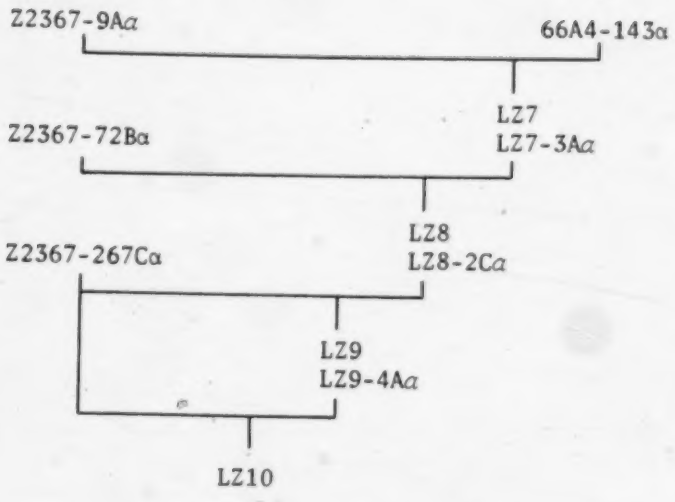
(c) Pedigree of LZ13

Genotypes of haploid strains:

Z2367-619C	*a*	*his1-1 arg6 trp2 ade1*
LZ4-65D	α	*hom3 his1-315 ade1*

FIGURE 5 cont'd.

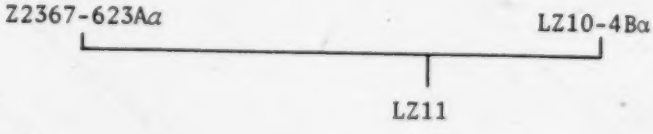
(d) Pedigree of LZ10



Genotypes of haploid strains:

Z2367-9A	α	*ura3 hom3 his1-1 le1 ade1 ade6*
Z2367-72B	α	*ura3 hom3 arg6 trp2 ade1 ade6*
Z2367-267C	α	*ura3 hom3 his1-1 le1 ade1*
66A4-143	α	*his1-30*
LZ7-3A	α	*his1-30 ade6 (ade1?)*
LZ8-2C	α	*his1-30 arg6 trp2 ade6 ade1*
LZ9-4A	α	*his1-30 arg6 trp2 ade6 ade1*

(e) Pedigree of LZ11



Genotypes of haploid strains:

Z2367-623A	α	*his1-315 arg6 trp2 le1 ade1*
LZ10-4B	α	*ura3 hom3 his1-30,1 ade6 ade1*

depending upon the approximate initial concentration of asci. Samples of the suspension were then streaked onto appropriate plates in readiness for dissection.

Unselected Tetrad Dissections

After incubation in the glusulase mixture, asci were streaked onto YEPD plates and dissected with a Cailloux micromanipulator. The plates were incubated at 30°C for 2 to 3 days and then replica plated to appropriate omission media to determine auxotrophic requirements, which were ascertained after incubation at 30°C for 18-24 hours. Temperature sensitive *his1* alleles were confirmed by an additional replica on histidine$^-$ medium after incubation at room temperature (23°C) for 2 to 4 days.

Selected Tetrad Dissections

Although sporulation procedures were consistent for all five diploids the subsequent incubation period prior to dissection differed for the two and three point crosses. For all crosses, asci were streaked on histidine$^-$ medium.

Plates from the two point crosses were subsequently incubated at room temperature for 10-12 hours before dissection, to allow for growth of prototrophic spores.

In the three point crosses the nature of the project was to treat the ts alleles as silent markers and therefore prototrophic spores could be of two kinds, those which carry a ts allele and those which carry its wild type allele at the *his1* locus. To minimize the differences in growth rate of these two types, the plates were incubated at 19°C for 34-36 hours. This enabled an unbiased selection of prototrophic spore-containing tetrads.

After incubation and before dissection of selected tetrads, 0.25 ml of histidine stock solution (2 mg/ml) was pipetted onto the center of the plate, away from the streak of asci, and allowed to diffuse into the medium. This converted the plate into an MC plate. Asci containing budded ascospores could then be dissected directly on the plate as for unselected tetrad dissections. Included in the selection were cases where the prototrophic spore had divided two or three times prior to dissection. These asci were easily discernible from groups or clumps of non-prototrophic spores.

The plates were then incubated at 30°C for 2-3 days and then replica plated to YEPD and histidine$^-$ media. Appropriate tetrads--those with not less than two viable non-prototrophic spores and at least one prototrophic spore--were transferred to YEPD and subsequently analyzed by replica plating for auxotrophic requirements.

Allele Identification

The identity of *his1* alleles in the dissected spores was determined by crossing clones of each spore to appropriate tester strains and checking for histidine prototrophy resulting from mitotic recombination between the unknown *his1* allele of the spore clone and the *his1* of the tester. There would be no histidine prototrophs produced if the spore clone contained the same allele as the tester.

The method was as follows. Plates of dissected asci were replica plated onto twice as many YEPD plates as alleles represented in the diploid. Suspensions of each relevant tester strain (both mating types) were prepared. Initially, these were applied, drop by drop, to each colony on a YEPD replica plate using a 1 ml syringe. In later experiments the tester strains were applied by spraying with a 100 ml atomizer.

The plates were then incubated overnight at 30°C and then replica plated to adenine$^-$ medium, which selects for diploids, in which *ade1-1* and *ade2-1* will complement. In addition to selecting for diploids, the mating type of individual colonies can be identified at this stage. The diploid bearing plates were then replica plated to histidine$^-$ medium, incubated at 30°C for 3 to 4 days and then examined for histidine prototrophy. Spores containing the same *his* mutant as the tester strain did not produce prototrophic colonies. Ambiguous results were repeated, at which time mitotic recombination was enhanced by exposure to 20 sec. of U.V. light (dose rate = 14 ergs/mm^2/sec) immediately after the replica plating to histidine$^-$ medium.

This procedure was later modified to include routine U.V. exposure. Spore colonies were mated as before on YEPD and after 24 hrs incubation were replica plated to low histidine, adenine$^-$ medium. The plates were then exposed to U.V. light for 20 seconds and incubated at 30°C for 2-3 days before examination for *his* prototrophic colonies. This method produced very few ambiguous results.

This technique was relatively unsatisfactory for crosses involving allele *his1-30*, which has a high induced homoallelic reversion rate (Korch and Snow 1973). The U.V. treatment therefore made scoring difficult. Thus, in tests involving *his1-30*, matings were made as before, on YEPD, allowing 1-2 days growth or replica plating to adenine$^-$ media to insure diploid formation. The diploids were then replica plated to sporulation plates and six days later the F^+ plates were replicated to histidine$^-$ plates and allowed to grow. The relatively high rate of meiotic recombination produced by this procedure gave unambiguous results in these crosses.

RESULTS

Unselected Tetrad Analysis

The analysis of 8679 unselected tetrads from five closely related diploid strains are presented here. These diploid strains include Z2367, LZ4, LZ6, LZ10 and LZ11; unselected tetrads were not dissected from the control strain LZ13. Each diploid is represented by approximately 1600 complete four-spored tetrads. Tetrads in which more than two markers showed aberrant segregation patterns are not included in these results as they were judged to be possible false tetrads. Ascus formation on F^+ medium was consistent in all five diploids. Complete tetrad survival on YEPD was also consistent and was greater than 80%. All tetrads were scored for all markers. Only complete tetrads, however, are included in the following analysis. Although the main emphasis of this study is on recombination at the *his1* locus, unselected tetrads also provide information on mapping linkage group V, interference and conversion patterns of other markers.

Intergenic Recombination and Mapping in Linkage Group V

The markers of linkage group V used in this study are *uracil 3* (*ura3*), *homoserine 3* (*hom3*), *histidine 1* (*his1*), *arginine 6* (*arg6*) *tryptophan 2* (*trp2*). Mortimer and Hawthorne (1966) have given the order as above with the centromere located between *ura3* and *hom3*, 5 centimorgans from *ura3* and 34 centimorgans from *hom3*. The mapping data for the remaining markers based on the results of the unselected tetrad dissections of the 5 diploids is presented in Table 2. Map distance was calculated using the formula map distance = $\frac{T + 6NPD}{2N} \times 100\%$. The results from Table 2 indicate no gross discrepancies in intergenic recombination in linkage group V among the five strains and are consistent

TABLE 2. Tetrad analysis for linkage in Group V.

Diploid Strain	INTERVAL									MAP DISTANCE		
	hom3 - his1			*his1 - arg6*			*arg6 - trp2*			*hom3 - his1*	*his1 - arg6*	*arg6 - trp2*
	PD	NPD	T	PD	NPD	T	PD	NPD	T			
Z2367	1543	0	94	1409	1	227	982	9	646	2.9	7.1	21.4
LZ4	1582	0	69	1446	1	204	1020	6	625	2.1	6.4	20.0
LZ10	1605	2	76	1438	1	244	1044	4	635	2.6	7.4	19.7
LZ6	1776	1	98	1627	0	248	1203	3	669	2.8	6.6	18.3
LZ11	1751	0	82	1584	1	248	1112	7	714	2.2	6.9	20.6
TOTAL	8257	3	419	7504	4	1171	5361	29	3289	2.5	6.8	20.0

with previous studies. The same intervals mapped by Mortimer and Hawthorne (1966) give the map distance from *hom3* to *his1* as 3.6, *his1* to *arg6* as 6.25, and *arg6* to *trp2* as 17.07. Another study (Fogel and Hurst 1967) using a larger sample, report the map distance from *hom3* to *his1* as 2.4, *his1* to *arg6* as 10.0, and *arg6* to *trp2* as 20.8.

Crossover and Chromatid Interference in Linkage Group V

Muller (1916) defined genetical interference as the phenomenon whereby the occurrence of one crossover interferes with the coincident occurrence of another in the same linkage group. The intensity of the interference is measured as the coincidence C which is equal to the $\frac{\text{observed no. of double exchanges}}{\text{expected no. of double exchanges}}$. C is generally found to be distance dependent and if the distance is great enough (35%) interference disappears (C=1). Table 3 gives the number of observed double crossovers and indicates the number expected based on the exchange frequency $\frac{(T + 6NPD)}{N}$ calculated from Table 2. The expected number for a particular interval is the product of the total asci and the single exchange frequencies for the component intervals and assumes no interference. The ratio of the observed to the expected gives the coincidence value for each pair of regions. The coincident values for the total results are 0.25 for the *hom-his*, *his-arg* region, 0.41 for the *his-arg*, *arg-trp* region and 0.43 for the *hom-his*, *arg-trp* region. Chiasma interference is observed in these data with the interference being stronger over the shorter distances.

Chromatid interference is the term given by Mather (1933) to the phenomenon of non-random involvement of the chromatids engaged in successive crossovers. If involvement is random, one expects a ratio of

TABLE 3. Coincidence of double exchanges within the *hom3 - trp2* interval.

Diploid Strain	Total Asci	Observed number/Expected number of tetrads PAIRED INTERVAL *hom-his, his-arg*	*his-arg, arg-trp*	*hom-his, arg-trp*
Z2367	1637	$\frac{4}{13.4} = .30$	$\frac{36}{99.7} = .36$	$\frac{17}{40.2} = .42$
LZ4	1651	$\frac{0}{8.8} = .0$	$\frac{34}{84.1} = .40$	$\frac{16}{27.6} = .58$
LZ10	1683	$\frac{3}{13.1} = .23$	$\frac{46}{98.2} = .47$	$\frac{11}{34.5} = .32$
LZ6	1875	$\frac{5}{13.7} = .36$	$\frac{34}{90.8} = .37$	$\frac{21}{38.1} = .55$
LZ11	1833	$\frac{3}{11.4} = .26$	$\frac{44}{104.8} = .42$	$\frac{10}{33.9} = .29$
TOTAL	8679	$\frac{15}{60.2} = .25$	$\frac{194}{476.5} = .41$	$\frac{75}{174.5} = .43$

1:2:1 for two-strand doubles to three-strand doubles to four-strand doubles. There is some evidence from studies in *Neurospora crassa* (Bole-Gowda, Perkins and Strickland 1962) that two-strand double crossovers show significant excess over four-strand double crossovers, whereas other studies, (Knapp and Moller 1965, Strickland 1958, and Ebersold and Levine 1959; cited by Catcheside 1977) show no chromatid interference.

The relevant data from this study are presented in Table 4 and these results do show chromatid interference with a significant excess of two-strand doubles over four-strand doubles, $\chi^2 = 10.39$, $p < .01$.

Conversion Patterns in One Point Crosses

Unselected tetrad analysis of the five diploids provided information as to the conversion frequency and pattern at six different loci heterozygous for a single mutant. These data are presented in Table 5. The mating type locus is also included in certain of the crosses. Conversion of mutant to wild type and wild type to mutant generally appears to be equal for most alleles as has been found in other studies of *Saccharomyces cerevisiae* (DiCaprio 1976; Fogel and Mortimer 1969). This observation has been termed parity of gene conversion. Five of the six alleles tested here do not depart significantly from parity; however, *trp2* shows significantly more total conversion to wild type than to mutant and this disparity is evident in all diploids. It is possible that this reflects a real bias in the direction of correction of mismatch. However, it was noted during the study that the *trp2* mutant was a leaky mutant and it is possible that a spore showing the apparent conversion to wild type may still be mutant. Allele scoring was based on replica plating to omission medium and the genotype was not further resolved

TABLE 4. Strand relationships of double exchanges.

Diploid Strain	*hom-his, his-arg* 2S, 3S, 4S			Paired Intervals *his-arg, arg-trp* 2S, 3S, 4S			*hom-his, arg-trp* 2S, 3S, 4S			Total 2S, 3S, 4S		
Z2367	0	2	2	13	17	6	4	9	4	17	28	12
LZ4	0	0	0	7	21	6	4	10	2	11	31	8
LZ10	1	1	1	18	22	6	2	4	5	21	27	12
LZ6	3	2	0	12	18	4	7	10	4	22	30	8
LZ11	2	1	0	8	27	9	7	2	1	17	30	10
TOTAL	6	6	3	58	105	31	24	35	16	88	146	50

Observed total 2S, 3S, 4S = 88, 146, 50

Expected total to fit 1:2:1 ratio = 71, 142, 71

$X^2 = 10.39$

$p < .01$

TABLE 5. Segregation patterns of other alleles.

Allele		DIPLOID STRAIN Z2367	LZ4	LZ10	LZ6	LZ11	Total 3:1/1:3	Percent Conversion
ura3	3:1	2	10	4	7	4	27:20	.54
	1:3	1	3	7	6	3		
hom3	3:1	5	5	4	8	2	24:17	.47
	1:3	4	6	5	2	0		
arg6	3:1	3	1	2	6	3	15:23	.44
	1:3	9	1	6	5	2		
trp2	3:1	7	11	8	7	7	40:12	.60
	1:3	1	3	1	3	4		
leu1	3:1	4	6	9	7	7	33:32	.75
	1:3	4	4	11	4	9		
ade6	3:1	1	0	2	5	3	11:10	.24
	1:3	3	2	2	2	1		
a/α	3α:1α	4	not		4	5*	13:7	.37
	3α:1α	2	tested		3	3		

by genetic allele identification tests. It is also possible that the apparent increase in conversion to wild type is the result of a lethal event associated with conversion to mutant. An analysis of incomplete tetrads showed no evidence of selection against tryptophan auxotrophs.

Intragenic Recombination at the *his1* Locus Among Unselected Tetrads

Of the 8,679 unselected tetrads analyzed during this study, a total of 205 tetrads (2.4%) provided evidence of a recombinational event at the *his1* locus. The description and numbers of each type of tetrad showing intragenic recombination at *his1* are presented in Table 6. Not included in the table are tetrads which show post-meiotic segregation and tetrads in which the recombinational event within the *his1* gene requires conversion on more than one chromatid. There were a total of four such tetrads among the unselected sample and these exceptional asci, as well as those showing post-meiotic segregation, are described in Table 11.

Polarity Pattern and Establishment of the Map Position of the Temperature Sensitive Alleles

Polarity of conversion is evident from the data described in Table 6. In all crosses the proximal allele, whether *his1-315* or *his1-30* undergoes conversion more often than the distal allele. This reflects a polarity of conversion, high to the proximal end, low to the distal end, and is consistent with a previous study of gene conversion at the *his1* locus (Fogel and Hurst 1967).

The pattern of double site conversion of *his1-30* in the three point cross, LZ11 (*315* x *30.1*) shows that *his1-30* was co-converted with either the proximal allele *his1-315* or the distal allele *his1-1* and no instance

TABLE 6. Description of asci containing a recombinational event within the *his1* locus. These asci were isolated from unselected tetrad dissections. (Numbers in brackets indicate the number of conversion events which included the temperature sensitive site.)

Ascus Class	Spore 1	Spore 2	Spore 3	Spore 4	DIPLOID STRAIN Z2367	LZ4	LZ10	LZ6	LZ11	Total
1	+ ++ - -	b	a	b	2	4	1	6	1(1)	14
2	+ ++ - -	b	+ m_1+ - +	- +m_2 + -	3	3	0	2	1	9
3	+ ++ - -	b	+ m_1+ + +	- +m_2 - -	1	0	0	2	0	3
4	+ ++ - +	- +m_2 + -	a	b	1	0	0	0	0	1
5	+ ++ + +	- +m_2 - -	a	b	0	0	0	1	1(1)	2
6	+ ++ + -	- +m_2 - -	+ m_1+ - +	b	0	0	0	0	1	1
7	- ++ - -	+ +m_2 + +	a	b	5	5	0	4	4(2)	18
8	- ++ - +	+ +m_2 + -	a	b	0	1	0	1	0	2
9	- ++ - -	+ +m_2 + +	+ m_1+ - +	- +m_2 + -	0	1	0	0	0	1
10	- ++ - -	+ +m_2 - -	+ m_1+ + +	b	0	0	0	1(1)	0	1
11	- ++ + +	+ +m_2 - -	a	b	0	0	1	0	0	1
12	- m_1m_2 + +	a	a	b	4	3	3	5	3(2)	18
13	- m_1m_2 + +	a	+ m_1+ - +	- +m_2 + -	3	0	0	0	0	3
14	- m_1m_2 + +	a	+ m_1+ + +	- +m_2 - -	0	1	0	0	1	2
15	- m_1m_2 + -	+ m_1+ - +	a	b	3	0	1	0	1(1)	5
16	- m_1m_2 + -	+ m_1+ - +	+ m_1+ - +	- +m_2 + -	0	1	0	0	0	1
17	- m_1m_2 + -	+ m_1+ - +	+ m_1+ + +	- +m_2 - -	0	0	0	0	1(1)	1
18	- m_1m_2 - -	+ m_1+ + +	a	b	6	1	0	2	1	10
19	- m_1m_2 - +	+ m_1+ + -	a	b	1	0	0	0	0	1
20	+ m_1m_2 + +	- m_1+ - -	a	b	4	1	1	1	1(1)	8
21	+ m_1m_2 + +	- m_1+ + +	a	- +m_2 - -	1	0	0	0	0	1
22	+ m_1m_2 + +	- m_1+ - +	a	- +m_2 + -	0	0	0	1	0	1
	Total Proximal Conversions				34	21	7	26	16	104

TABLE 6 (cont'd)

Ascus Class	Spore 1	Spore 2	Spore 3	Spore 4	DIPLOID STRAIN Z2367	LZ4	LZ10	LZ6	LZ11	Total
50	- ++	- -	+ m_1m_2	+ +	a	b	1	1	0	0	0	2
51	- ++	+ +	+ m_1m_2	- -	+ m_1+	- + - +m_2	0	1	0	0	0	1
	Total Reciprocal Events						1	2	0	0	0	3
	Exceptional Asci						0	1	2	1	0	0
Total event asci							54	45	14	59	34	206
Total tetrads							1637	1651	1683	1875	1833	
Percent asci with recombination event							3.3%	2.7%	0.8%	3.1%	1.9%	

All crosses are of the general form $\frac{+ \quad m_1+ \quad arg6 \quad trp2}{hom3 \quad +m_2 \quad + \quad +}$ in which m_1 and m_2 are the proximal and distal alleles at the *his1* locus.

The *his1* marker combinations for the diploid strains are as follows:

Z2367	m_1 = *his1-315*	m_2 = *his1-1*
LZ4	m_1 = *his1-315*	m_2 = *his1-1s*
LZ10	m_1 = *his1-30*	m_2 = *his1-1*
LZ6	m_1 = *his1-315*	m_2 = *his1-1*
LZ11	m_1 = *his1-315*	m_2 = *his1-1*

LZ6 and LZ11 each have an additional site of heterozygosity, which is temperature sensitive, between m_1 and m_2. Conversion at m_1 or m_2 does not necessarily result in co-conversion of the temperature sensitive marker. Spores in which co-conversion of the temperature sensitive marker did occur are indicated in brackets. There were no instances of single site conversion of the temperature sensitive markers.

"a" indicates + m_1+ - - "b" indicates - +m_2 + +

23	+ m_1m_2 - -	b	a	b	1	0	0	0	0	1
24	+ m_1m_2 - +	- $+m_2$ + -	a	b	0	1	0	0	0	1
25	+ m_1m_2 + +	- $+m_2$ - -	a	b	1	0	0	1(1)	2	4
26	+ m_1m_2 + +	- $+m_2$ - -	+ m_1+ - +	- $+m_2$ + -	0	0	0	0	1	1
27	+ m_1m_2 + -	- $+m_2$ - -	+ m_1+ - +	b	1	0	0	0	0	1
28	+ m_1m_2 + -	- $+m_2$ - +	a	b	0	0	0	0	1(1)	1
29	- m_1m_2 - -	+ $+m_2$ + +	a	b	2	0	0	0	0	2
30	- ++ + +	a	a	b	0	1	1	0	0	2
31	- ++ + -	+ m_1+ - +	a	b	0	0	0	1(1)	0	1
32	- ++ - -	+ m_1+ + +	a	b	0	1	0	2(2)	1	4
33	+ ++ + +	- m_1+ - -	a	b	0	0	1	0	0	1
34	+ ++ - -	- m_1+ + +	a	b	0	0	0	1(1)	0	1
	Total Distal Conversions				5	3	2	5	5	20
35	+ $+m_2$ - -	b	a	b	4	2	1	3(3)	1(1)	11
36	+ $+m_2$ - -	b	+ m_1+ - +	- $+m_2$ + -	0	2	0	1(1)	1(1)	4
37	+ $+m_2$ - -	b	+ m_1+ + -	- $+m_2$ - +	1	0	0	0	0	1
38	+ $+m_2$ - +	- $+m_2$ + -	a	b	1	2	0	2(2)	2(2)	7
39	+ $+m_2$ - -	- $+m_2$ - -	- m_1+ + +	+ $+m_2$ + +	0	1	0	0	0	1
40	+ $+m_2$ + +	- $+m_2$ - -	a	b	3	6	0	3(3)	1(1)	13
41	+ $+m_2$ + -	- $+m_2$ - +	a	b	0	1	0	1(1)	0	2
42	+ $+m_2$ + -	- $+m_2$ - -	+ m_1+ - +	b	1	2	0	0	0	3
43	+ $+m_2$ + +	- $+m_2$ - -	+ m_1+ - +	- $+m_2$ + -	0	0	1	0	0	1
44	- m_1+ + +	a	a	b	0	1	1	6(6)	3(3)	11
45	- m_1+ + +	a	+ m_1+ - +	- $+m_2$ + -	0	0	0	2(2)	1(1)	3
46	- m_1+ + -	+ m_1+ - +	a	b	0	0	0	1(1)	1(1)	2
47	- m_1+ - -	+ m_1+ + +	a	b	2	1	0	7(7)	2(2)	12
48	- m_1+ - -	+ m_1+ + +	+ m_1+ - +	- $+m_2$ + -	1	0	0	0	0	1
49	- m_1+ - +	+ m_1+ + -	a	b	1	0	0	1(1)	1	3
	Total Co-conversions				14	18	3	27	13	75

of co-conversion of these two markers was found without co-conversion of *his1-30*. This suggests that *his1-30* is located between *his1-315* and *his1-1*. The observation that *his1-30* was co-converted more often with *his1-315* than with *his1-1* (9/16 of proximal conversions : 1/5 of distal conversions) suggests that *his1-30* is closer to *his1-315*. This is in contrast to the work of Korch and Snow (1973) who suggest that *his1-30* was close to but proximal to *his1-315* based on data from X-ray mapping. Korch and Snow (1973) describe the location of *his1-1s* as distal to *his1-315* and proximal and close to *his1-1*. The data presented in Table 6 are consistent with this location. Thus, in the three-point cross, LZ6, the additional "silent" marker, *his1-1s*, is relatively distal; whereas in the other three-point cross, LZ11, the additional "silent marker, *his1-30*, is relatively proximal.

Asymmetry of Recombination at his1

The overwhelming majority of conversion events analyzed seem to be confined to a single chromatid. Determination of the chromatid involved in any event depends on an intact m_1+ chromatid, an intact $+m_2$ chromatid, and a third intact (either $+m_2$ or m_1+) chromatid. Table 7 summarizes the data on unselected tetrads from Tables 6 and 11 on this basis and further describes the event as proximal or distal conversion, or as co-conversion of the involved markers. In four of the five crosses, the ratio of chromatids which underwent conversion, $+m_2$: m_1+, is approximately 1:1. Tetrads from LZ4 (*315* x *1s*) show a substantial departure from this equality, with the m_1+ strand being more frequently converted.

The pattern of detectable events on the m_1+ strand is consistent between the five diploids; co-conversion is approximately equal to

TABLE 7. Summary of recombination events on the m_1+ and $+m_2$ chromatids at the *his1* locus. (Numbers in brackets indicate co-conversion of temperature sensitive markers.)

Strand	Event	DIPLOID STRAIN Z2367	LZ4	LZ10	LZ6	LZ11	Total
m_1+	$+m_2$ (co-conversion)	10	16	2	10(10)	5(5)	43
	++ (proximal conversion)	12	14	2	17(1)	8(4)	53
	m_1m_2 (distal conversion)	5	1	0	1(1)	4(1)	11
	post-meiotic segregation	0	0	1	0(0)	0(0)	1
	Total	27	31	5	28	17	108
$+m_2$	m_1+ (co-conversion)	4	2	1	17(17)	8(8)	32
	m_1m_2 (proximal conversion)	22	7	5	9	8(5)	51
	++ (distal conversion)	0	2	2	4(4)	1	9
	post-meiotic segregation	0	1	0	0	0	1
	Total	26	12	8	30	17	93
	Reciprocal event	1	2	0	0	0	3
	2 strand event	0	0	1	1(1)	0	2
	Total Events	54	45	14	59	34	206

proximal conversion and both are much more frequent than distal conversion. On the $+m_2$ strand, results differ between the two- and three-point crosses. In the two-point crosses, proximal conversion predominates over both co-conversion and distal conversion. However, in the three-point crosses, the pattern of events is much the same as on the m_1+ strand. The inclusion of the "silent" markers has the apparent effect of altering events on the $+m_2$ strand by "extending" the proximal conversion event ($+ \rightarrow m_1$) to include the distal allele in a co-conversion event ($+m_2 \rightarrow m_1+$). The proximal event on the opposite strand appears to be unaffected by the addition of a silent marker.

The relative amount of co-conversion of *his1-315* and *his1-1* in detectable events in the two-point cross was compared with each of the three-point crosses. Heterogeneity chi-square analysis indicated that the increase in co-conversion detected in LZ6 (*315.1s* x *1*) was significant ($\chi_1^2 = 4.8$, $p < .05$).

Conversion Frequencies

The frequency of recombination events and the conversion frequencies of the alleles in each diploid strain are given in Table 8. Two frequencies for each allele in each strain have been determined. One is the "basic conversion frequency" calculated as the detected frequency of conversion per tetrad, and the other is the frequency of conversion per *his1* recombinant tetrad. Variations in the basic conversion frequency for an allele between strains might reflect changes in the conversion patterns at the locus, changes in the recombination frequency at the locus, or changes in the overall recombination rate in the strain. Changes in the frequency of conversion per recombinant tetrad reflect

TABLE 8. Conversion frequencies of *his1-315*, *his1-1s*, *his1-30*, and *his1-1* in five diploid strains.

TYPE OF CONVERSION	DIPLOID STRAIN Z2367		LZ4		LZ10		LZ6			LZ11		
	allele *315*	*1*	allele *315*	*1s*	allele *30*	*1*	allele *315*	*1s*	*1*	allele *315*	*30*	*1*
m → +	22	4	30	4	4	2	26	12	21	13	13	10
+ → m	26	15	9	17	6	4	25	21	11	16	9	8
Total	48	19	39	21	10	6	51	33	32	29	22	18
Total recombinant tetrads	54		45		14		59			34		
Total tetrads	1637		1651		1683		1875			1833		
Event frequencies	.033		.027		.008		.031			.019		
Percent conversion frequency per recombinant tetrad	87%	35%	87%	47%	71%	43%	86%	56%	54%	85%	65%	53%
Percent conversion frequency per total tetrads	2.9%	1.1%	2.4%	1.2%	0.6%	0.4%	2.7%	1.8%	1.7%	1.6%	1.2%	1.0%

much more specifically changes in the conversion patterns at the locus.

The data presented in Table 8 suggest that conversion frequencies of alleles used in this study may differ depending upon whether they are included in two-point crosses or three-point crosses. Accordingly, the data were tested for heterogeneity and the result of this analysis is shown in Table 9.

A comparison of the standard cross Z2367 (*315* x *1*) with the three-point cross LZ11 (*315.30* x *1*) indicates that the proportion of tetrads containing a recombination event at *his1* (event frequency) is significantly reduced in the three-point cross ($\chi^2_1 = 7.3$, $p < .01$). The basic conversion frequency of *his1-315* is also significantly reduced ($\chi^2_1 = 7.26$, $p < .01$). One suggestion is that the presence of the extra site of heterozygosity, *his1-30*, causes a decrease in the conversion of the proximal allele, thereby decreasing the event frequency. To decide if this decrease is due to an extra site of proximal heterozygosity or is due to the nature of *his1-30 per se*, LZ10 (*30* x *1*) in which *his1-30* is present in a two-point cross, was considered. The presence of *his1-30* in this cross is also accompanied by a reduction in event frequency. This reduction is significantly greater than that observed in LZ11 (*315* x *30.1*) ($\chi^2_1 = 6.8$, $p < .05$), moreover the basic conversion frequency of *his1-30* is less in the two-point cross than in the three-point cross. Thus it may be suggested that *his1-30* has a low basic conversion frequency and that it can impose this "pattern" on *his1-315* in the three-point cross. The fact that its own low frequency, as determined in LZ10 (*30* x *1*), is increased in the three-point cross suggests that a marker interaction of *his1-315* and *his1-30* results in alterations to the conversion frequencies of both alleles in the three-point cross.

TABLE 9. Heterogeneity chi-square analysis of conversion frequencies from Table 8.

	Strains Compared	Conversion per Tetrad		Conversion per Recombinant Tetrad	
		χ^2	df	χ^2	df
CONVERSION:					
his1-315	Z2367, LZ4, LZ6, LZ11	8.08*	3	0.10	3
	Z2367, LZ6	0.14	1	0.16	1
	Z2367, LZ11	7.26**	1	0.31	1
his1-1	Z2367, LZ6, LZ10, LZ11	15.32**	3	4.60	3
	Z2367, LZ6	1.82	1	4.13*	1
	Z2367, LZ11	0.26	1	2.70	1
	Z2367, LZ10	7.18**	1	0.28	1
his1-1s	LZ4, LZ6	1.78	1	1.34	1
his1-30	LZ10, LZ11	3.57	1	0.20	1
EVENT FREQUENCY:					
	Z2367, LZ4, LZ6, LZ10, LZ11	30.00**	4		
	Z2367, LZ4, LZ6	1.02	2		
	Z2367, LZ11	7.29**	1		
	LZ10, LZ11	6.80**	1		

* $p<0.05$
** $p<0.01$

A comparison of the standard cross Z2367 (*315* x *1*) with the other three-point cross, LZ6 (*315.1s* x *1*), indicates that the conversion frequency of *his1-1* per recombinant tetrad is significantly increased in the three-point cross ($\chi^2 = 4.13$ $p < .05$). The suggestion is that the pattern of recombination in the three-point cross is altered by the presence of an extra site of heterozygosity such that the increase in co-conversion as noted in the previous section is accompanied by an actual increase in the conversion frequency of *his1-1*. However, although an increase in the basic conversion frequency of *his1-1* is seen in the three-point cross it was not shown to be significant.

Flanking Marker Configuration of his1 Recombinant Spores

There are four possible configurations of the flanking markers, *hom3* and *arg6*, which can be associated with the *his1* recombinant spore. These are referred to as P1, P2, R1, and R2. Generally this classification has been used to describe prototrophic spores. In this study both prototrophic and double mutant spores are considered and this results in there being two versions of each of the four classes. Thus, given the diploid: $\frac{- \; +m_2 \; +}{+ \; m_1 + \; -}$

P1 refers to the parental configuration of flanking markers which entered the cross with the proximal allele. The P1 configuration of flanking markers for a conversion event which gives rise to a prototroph is + ++ -, and the P1 configuration of flanking markers for a conversion event which gives rise to double mutant spores is - $m_1 m_2$ +.

P2 refers to the parental configuration of flanking markers which entered the cross with the distal allele. The P2 configuration for a prototrophic spore is - ++ +, and for a double mutant spore is + m_1m_2 -.

R1 refers to the recombinant configuration of flanking markers that would result from a single crossover between the alleles or a conversion event with an adjacent crossover. The R1 configuration for a prototrophic spore is - ++ -, and is + m_1m_2 + for a double mutant spore.

R2 refers to recombinant configuration of flanking markers that would result from a triple exchange, or more simply, from conversion of one allele separated from a crossover by a region containing the second unconverted allele. The R2 configuration for a prototrophic spore is + ++ + and for a double mutant spore is - m_1m_2 -. In general, the R2 configuration is less common than the R1 configuration.

Two hundred and two *his1* convertants are described in Table 6, of which 105 have parental combinations of the flanking markers, *hom3* and *arg6*, and 97 are recombined for these markers. This reflects a high degree of association of gene conversion and recombination of the flanking markers.

There are 104 tetrads which show proximal allele conversion at *his1* (Table 6, classes 1-22) and of these, 46 are recombinant for flanking markers. Thirty two show the R1 configuration (Table 6, classes 7-10 and 20-22) and might originate from a crossover on either side of the proximal converted allele. If the crossover was distal to the proximal

allele then it would be within the *his1* locus and proximal to the distal marker. The remaining 14 tetrads show the R2 configurations (Table 6, classes 5, 6, 18 and 19). The position of the crossover in these tetrads is less ambiguous. They probably originate from a crossover distal to the unconverted distal allele, in the region between *his1* and *arg6*. If exchange in the *his1* to *arg6* region is not related to the proximal conversion and if, as was suggested by Stadler (1959) and Mortimer and Fogel (1974), conversion alone produces no interference, it is possible to calculate the expected exchange frequency in this region. Thus in a total of 70 tetrads (non-R1) using the map distance of 6.88 (Table 1), one expects 9.6 tetrads with an exchange in the *his1* to *arg6* region. Assuming random chromatid involvement, it would be expected that one-half of these would be associated with a conversion event. The data show that 22 tetrads with a proximal conversion have an exchange in the *his1* to *arg6* region. Fourteen of these exchanges involve the converted chromatid and eight do not (Table 6, classes 3, 10, 14, 17 and 21). The excess of exchanges in this region and, particularly, the almost three-fold excess of exchanges associated with the proximal conversion suggests that the two events are not always independent.

Of the 20 distal convertant tetrads there are 14 which are recombinant for flanking markers. Eleven of these are the R1 configuration (Table 6, classes 25, 28 and 32) and three are of the R2 configuration (Table 6, classes 29 and 33). These three have a crossover in the proximal region, *hom3* to *his1*. There were no tetrads with unrelated crossovers in this region among the distal convertants. Further discussion of R2 tetrads with distal conversion will follow in the section on selected tetrads.

There are 75 tetrads showing co-conversion of the outermost alleles and of these 35 are recombinant for flanking markers. In these events the position of the exchange which resulted in the recombination of flanking markers cannot be determined.

Finally, although the overall ratio of parental to recombinant configuration of flanking markers is approximately 1:1, the ratios vary with the different types of conversion events. The ratio of P:R for proximal conversion events is 1:.79, for co-conversion events is 1:.88 and for distal conversion events is 1:2.33. The significance of this is not obvious at present.

Selected Tetrad Analysis

Table 10 presents the results and classification of the dissection of 1110 selected tetrads. The tetrads represented here include only asci in which all four spores were viable and which contain one prototrophic spore. Tetrads were selected at restrictive temperature for the two point crosses and at permissive temperature for the three point crosses. Thus, in the three point crosses the prototrophic spore may be temperature sensitive histidine$^-$. In Table 10 conversion of the temperature sensitive site is indicated in brackets for diploids LZ6 and LZ11.

For diploid LZ6 $(\frac{315\ 1s\ +}{+\ \ +\ 1})$ co-conversion of the temperature sensitive site (*1s*) with the proximal allele results in a non-temperature sensitive prototroph + + +. Single site conversion of the proximal allele results in a temperature sensitive prototroph + *1s* +. Co-conversion of the temperature sensitive allele with the distal allele results in a temperature sensitive prototroph + 1s + and single site conversion of the distal allele results in a non-temperature sensitive prototroph + + +.

TABLE 10. Description of *his* prototroph-spore-containing asci recovered from selected tetrad dissections of five diploid strains. (Numbers in brackets indicate co-conversion of temperature sensitive markers. See Table 6 for explanation of notation.)

Ascus Class	Spore 1	Spore 2	Spore 3	Spore 4	DIPLOID STRAIN Z2367	LZ4	LZ10	LZ6	LZ11	Total
1	- ++ - -	+ m_1m_2 + +	a	b	12	10	13	3	19	57
2	- ++ - -	+ m_1m_2 + -	+ m_1+ - +	b	0	2	2	1	2	7
3	- ++ - -	+ m_1m_2 + +	+ m_1+ - +	- +m_2 + -	0	1	0	0	0	1
4	- ++ - -	+ m_1m_2 + +	+ m_1+ + +	- +m_2 - -	0	0	1	0	0	1
5	- ++ - +	+ m_1m_2 + -	a	b	1	2	0	0	1	4
6	- ++ - +	+ m_1m_2 + +	a	- +m_2 + -	0	0	1	0	0	1
7	+ ++ - -	- m_1m_2 + +	a	b	1	0	0	0	0	1
8	+ ++ - -	- m_1m_2 - -	+ m_1+ + +	b	1	0	0	0	1	2
9	+ ++ - -	+ m_1m_2 + +	- m_1+ - -	b	0	1	0	0	0	1
10	+ ++ - -	- m_1m_2 + +	+ m_1+ - +	- +m_2 + -	1	0	0	0	1	2
	Total Reciprocal Events				16	16	17	4	24	77
11	+ ++ - -	b	a	b	57	70	27	70(2)	57(30)	281
12	+ ++ - -	b	+ m_1+ - +	- +m_2 + -	26	19	8	19	17(9)	89
13	+ ++ - -	b	+ m_1+ + +	- +m_2 - -	5	3	3	3(1)	5(2)	19
14	+ ++ - -	b	+ m_1+ + -	- +m_2 - +	1	0	0	0	0	1
15	+ ++ - -	b	- m_1+ - -	+ +m_2 + +	4	1	0	2	2	9
16	+ ++ - -	- +m_2 - +	+ m_1+ + +	- +m_2 + -	0	0	0	0	1(1)	1
17	+ ++ - +	- +m_2 + -	a	b	27	25	8	20(2)	18(13)	98
18	+ ++ - +	- +m_2 - -	+ m_1+ + -	b	1	0	0	0	0	1
19	+ ++ - +	+ +m_2 + -	- m_1+ - -	b	0	1	0	0	0	1
20	+ ++ - +	- +m_2 + -	+ m_1+ - +	- +m_2 + -	1	1	1	0	0	3
21	+ ++ + +	- +m_2 - -	a	b	19	15	11	27(2)	20(8)	92
22	+ ++ + +	- +m_2 - -	+ m_1+ - +	- +m_2 + -	2	1	0	0	2(1)	5
23	+ ++ + +	- +m_2 - +	+ m_1+ - -	- +m_2 + -	2	1	1	0	0	4
24	+ ++ + +	- +m_2 - -	+ m_1+ + +	- +m_2 - -	0	0	1	0	0	1

25	+ ++ + -	- $+m_2$ - +	a	b	2	1	1	1	0	5
26	+ ++ + -	- $+m_2$ - -	+ m_1+ - +	b	0	3	1	0	1(1)	5
27	- ++ - -	+ $+m_2$ + +	a	b	39	48	26	43(2)	37(15)	193
28	- ++ - -	+ $+m_2$ + -	+ m_1+ - +	b	3	3	0	1	3(1)	10
29	- ++ - -	+ $+m_2$ - -	+ m_1+ + +	b	2	1	0	0	0	3
30	- ++ - -	+ $+m_2$ + +	+ m_1+ + +	- $+m_2$ - -	1	0	0	0	0	1
31	- ++ - -	+ $+m_2$ + +	+ m_1+ - +	- $+m_2$ + -	4	4	1	0	4(2)	13
32	- ++ - +	+ $+m_2$ + -	a	b	3	4	2	2	5(2)	16
33	- ++ - +	+ $+m_2$ + +	a	- $+m_2$ + -	7	6	2	5	3(2)	23
34	- ++ - +	+ $+m_2$ + -	+ m_1+ - +	- $+m_2$ + -	0	1	0	0	0	1
35	- ++ + +	+ $+m_2$ - -	a	b	1	0	0	0	1(1)	2
36	- ++ + +	+ $+m_2$ - -	+ m_1+ - +	- $+m_2$ - -	0	0	0	0	2	2
	Total Proximal Conversions				207	208	93	193	178	879
37	- ++ + +	a	a	b	8	5	6	6(6)	5	30
38	- ++ + +	a	+ m_1+ - +	- $+m_2$ + -	4	4	1	6(5)	2	17
39	- ++ + +	a	+ m_1+ + +	- $+m_2$ - -	2	0	0	2(2)	0	4
40	- ++ + +	a	- m_1+ - -	+ $+m_2$ + +	0	1	0	0	1	2
41	- ++ + -	+ m_1+ - +	a	b	2	0	2	1(1)	5(3)	10
42	- ++ - -	+ m_1+ + +	a	b	12	7	19	6(4)	11(2)	55
43	- ++ - -	+ m_1+ + -	+ m_1+ - +	b	1	0	1	1	1	4
44	- ++ - -	+ m_1+ + +	+ m_1+ - +	- $+m_2$ + -	3	1	1	2(2)	0	7
45	- ++ - +	+ m_1+ + -	a	b	1	1	0	0	4(1)	6
46	- ++ - +	+ m_1+ + +	a	- $+m_2$ + -	1	0	2	1	2(1)	6
47	- ++ - +	+ m_1+ - +	+ m_1+ + -	- $+m_2$ + -	0	1	0	0	0	1
48	+ ++ + +	- m_1+ - -	a	b	0	2	2	0	2	6
49	+ ++ + +	- m_1+ - +	a	- $+m_2$ + -	0	0	1	0	0	1
50	+ ++ + -	- m_1+ - +	a	b	0	0	0	1	0	1
51	+ ++ + -	- m_1+ - -	+ m_1+ - +	b	0	0	0	0	1	1
52	+ ++ - -	- m_1+ + +	a	b	1	1	0	0	0	2
53	+ ++ - -	- m_1+ - -	+ m_1+ + +	b	0	0	1	0	0	1
	Total Distal Conversions				35	23	36	26	34	154
	Total tetrads				258	247	146	223	236	1110

For diploid LZ11 $(\frac{315\ +\ +}{+\ 30\ 1})$ co-conversion of the temperature sensitive site (*30*) with the proximal allele results in a temperature sensitive prototroph and single site conversion of the proximal allele results in a non-temperature sensitive prototroph + + +. Co-conversion of the temperature sensitive site with the distal allele results in non-temperature sensitive prototroph + + + whereas single site conversion of the distal allele results in a temperature sensitive prototroph + *30* +.

The rare asci containing two prototrophic spores or showing post-meiotic segregation are included in Table 11. Tetrads which have two or more loci showing aberrant segregation patterns are omitted. This was the criterion by which tetrads were judged to be false. It has been established (DiCaprio 1976) that long co-conversion lengths which can encompass more than one gene do occur in yeast and it is therefore possible that some tetrads were misclassified as false. Tetrads which may have represented co-conversion of two genes, however, were very rare.

Reliability of the Selection Technique

Selected tetrads were used in this study to increase the numbers of recombinational events available for analysis. The obvious disadvantage of this technique is that certain events such as co-conversion and conversion to mutant are not detected. An additional disadvantage is the possibility that there may be a bias in the selection system that is due to some influence other than the histidine independence of the recombinant spore.

A personal bias in the selection and dissection was not likely since one of the diploids, Z2367 was used in a previous study, Fogel and Hurst (1967) and the results of their selected tetrad data did not differ

TABLE 11. Description of exceptional tetrads, that is, tetrads showing post-meiotic segregation (pms) and/or tetrads in which conversion occurred on more than one chromatid.

EVENT	Spore 1	Spore 2	Spore 3	Spore 4	SOURCE
pms	- $\frac{(+\ 1s)}{(315\ +)}$ + +	- (+ 1s) + -	+ (315 +) - -	+ (315 +) - +	LZ4 unselected
pms	- (+ 1) + +	- (+ 1) + +	+ $\frac{(30\ 1)}{(30\ +)}$ - -	+ (30 +) - -	LZ10 unselected
pms	- (+ 1s) + +	- (+ 1s) + -	+ $\frac{(+\ +)}{(315\ +)}$ - +	+ (315 +) - -	LZ4 selected
pms	- $\frac{(+\ 30\ +)}{(+\ 30\ 1)}$ + -	- (+ 30 1) + +	+ (315 + +) - +	+ (315 + +) - -	LZ11 selected
pms	- (+ 30 1) + +	- (+ 30 1) + -	+ $\frac{(+\ +\ +)}{(315\ +\ +)}$ - +	+ (315 + +) - -	LZ11 selected
2 strand event with pms	- (+ 1) + +	- (+ +) - -	+ $\frac{(+\ +)}{(+\ 1)}$ + +	+ (30 +) - -	LZ10 unselected
2 strand event	- (+ + 1) + +	- (+ + +) - -	+ (+ + +) + +	+ (315 1s +) - -	LZ6 unselected
2 strand event	- (+ 1) + +	- (+ +) - -	+ (+ +) + +	+ (30 +) - -	LZ4 selected
2 strand event	- (+ 1s) + +	- (+ 1s) + +	+ (+ +) - -	+ (315 1) - -	LZ4 selected
2 strand event	- (+ 1s) + +	- (+ 1s) + -	+ (+ +) - -	+ (+ 1s) - +	LZ4 selected

significantly from the results presented here. Fogel and Hurst (1967) also established that spore death and histidine prototrophy were independent.

Bias may be encountered if one or more of the phenotypes associated with the other segregating linked loci have a selective advantage. For example, Mortimer and Fogel (1974) speculated that there may be a selective advantage associated with the wild-type allele of *hom3*. If this is the case then spores of the phenotype + ++ - (P1) and + ++ + (R2) would have been preferentially selected.

To examine this possibility a sixth diploid, LZ13, was constructed with the flanking markers in the alternative arrangement. Prototrophs from this diploid were selected and analyzed and the results are given in Table 12. To simplify comparisons, asci have been allocated class numbers indicating comparable genetic origins to those in Table 10, even though the genetic outcome reflects the initial dissimilarity of linkage phase.

To facilitate the comparison of flanking marker arrangement in this diploid with the others used in the study, the prototrophic spores shown in Tables 10 and 12 have been classified with respect to flanking marker configuration of the prototrophic spores recovered from the dissection of unselected tetrads. In Table 13 the configuration of flanking markers follows the P1, P2, R1, and R2 classification which was previously defined in the results section on unselected tetrad analysis. For all crosses, except LZ13, P1 is a + ++ - spore, P2 is a - ++ + spore, R1 is a - ++ - spore and R2 is a + ++ + spore. In the sample from LZ13, this arrangement is reversed so that P1 is - ++ +, P2 is + ++ -, R1 is + ++ + and R2 is - ++ -.

The two diploids Z2367 and LZ13, which have opposite marker arrangements yet carry the same *his1* alleles, were tested for homogeneity with

TABLE 12. Description of *his* prototroph-spore-containing asci recovered from selected tetrad dissections of LZ13.

Ascus Class	Spore 1	Spore 2	Spore 3	Spore 4	Total
1	+ ++ + +	- m_1m_2 - -	a	b	10
2	+ ++ + +	- m_1m_2 - +	- m_1+ + -	b	1
7	- ++ + +	+ m_1m_2 - -	a	b	1
	Total Reciprocal Events				12
11	- ++ + +	b	a	b	52
12	- ++ + +	b	- m_1+ + -	+ +m_2 - +	6
13	- ++ + +	b	- m_1+ - -	+ +m_2 + +	7
14	- ++ + +	b	- m_1+ - +	+ +m_2 + -	1
15	- ++ + +	b	+ m_1+ + +	- +m_2 - -	2
17	- ++ + -	- +m_2 - +	a	b	11
19	- ++ + -	- +m_2 - +	+ m_1+ + +	b	1
21	- ++ - -	+ +m_2 + +	a	b	12
25	- ++ - +	+ +m_2 + -	a	b	1
26	- ++ - -	+ +m_2 + -	a	+ +m_2 - +	1
27	+ ++ + +	- +m_2 - -	a	b	37
28	+ ++ + +	- +m_2 - +	- m_1+ + -	b	2
31	+ ++ + +	- +m_2 - -	- m_1+ + -	+ +m_2 - +	2
32	+ ++ + -	- +m_2 - +	a	b	2
34	+ ++ + -	- +m_2 - +	- m_1+ + -	+ +m_2 - +	1
35	+ ++ - -	- +m_2 + +	a	b	1
	Total Proximal Conversions				139
37	+ ++ - -	a	a	b	6
41	+ ++ - +	- m_1+ + -	a	b	1
42	+ ++ + +	- m_1+ - -	a	b	6
46	+ ++ + -	- m_1+ - -	a	+ +m_2 - +	1
	Total Distal Conversions				14
	Total Events				165

TABLE 13. Configuration of flanking markers of *his* prototrophic spores recovered from dissections of selected asci. The flanking marker configuration of the total *his* prototrophic spores isolated from dissections of unselected asci is also included.

Source of Prototrophic Spores	Flanking Marker Arrangement				Total Spores
	P_1	P_2	R_1	R_2	
Z2367 *(315)x(1)*	126	17	90	25	258
LZ13 *(315)x(1)*	81	8	62	14	165
LZ4 *(315)x(1a)*	122	10	92	23	247
LZ6 *(315 1a)x(1)*	114	15	65	29	223
LZ10 *(30)x(1)*	48	9	71	18	146
LZ11 *(315)x(30 1)*	102	16	92	26	236
Total excluding LZ13	512	67	410	121	1110
Total prototrophic spores from unselected tetrads	28	5	28	4	65

respect to the occurrence of P1, P2, R1, and R2 configurations. No significant heterogeneity was found ($\chi^2_3 = .896, p > .8$). In addition, pooled data from unselected tetrads were compared to the pooled data from selected tetrads (excluding LZ13). Again, no significant heterogeneity was found ($\chi^2_3 = 2.31, p > .5$). It would seem that flanking marker configuration did not bias the selection procedure.

A third factor which could contribute to a biased selected sample would be a differential selection of temperature sensitive prototrophs in the three-point crosses. Comparison of the frequency of the temperature sensitive prototrophs from the selected and unselected samples did not suggest that this had occurred. In diploid, LZ6, the frequency of temperature sensitive spores among total prototrophs in the unselected sample is 0.95 (20/21) and in the selected sample is 0.93 (208/223). In diploid LZ11 the frequency of temperature sensitive spores in the unselected prototrophs is 0.56 (5/9) and in the selected sample is 0.52 (123/236). Further comparisons of the data from selected and unselected tetrad dissections did not indicate any major discrepancies, as will be discussed later.

Proximal Allele Conversion

Tetrads which contained a prototrophic spore, two distal alleles and one proximal allele were defined as tetrads which had undergone conversion of the proximal allele. These asci are distributed in classes 11-36 of Tables 10 and 12. This category occurs most frequently for all diploids dissected, reinforcing the observation of strong proximal polarization in the unselected tetrads.

The frequency of proximal allele conversion for each diploid is presented in Table 14. Proximal allele conversion in the three-point cross may occur with or without co-conversion of the temperature sensitive

TABLE 14. Frequency of proximal allele conversion in selected and unselected tetrads.

Diploid Strain	SELECTED TETRADS	UNSELECTED TETRADS	
	conversion to + / total recombinants	conversion to + / total prototrophs	conversion to + or m / total recombinants*
Z2367 *(315)x(1)*	$\frac{207}{258} = .80$	$\frac{12}{13} = .92$	$\frac{34}{40} = .85$
LZ4 *(315)x(1a)*	$\frac{208}{247} = .84$	$\frac{14}{18} = .78$	$\frac{21}{26} = .81$
LZ10 *(30)x(1)*	$\frac{93}{146} = .64$	$\frac{2}{4} = .50$	$\frac{7}{9} = .78$
LZ6 *(315 1a)x(1)*	$\frac{193}{223} = .87$	$\frac{17}{21} = .81$	$\frac{26}{32} = .81$
LZ11 *(315)x(30 1)*	$\frac{177}{234} = .76$	$\frac{8}{9} = .89$	$\frac{14}{19} = .74$
LZ13 *(315)x(1)*	$\frac{139}{165} = .84$		

* "total recombinants" does not include co-conversion events which encompassed all the markers in the gene

allele. For comparison, Table 14 includes the frequencies of proximal allele conversion among prototrophs recovered from the dissection of unselected tetrads. Comparisons of the two frequencies for each diploid using heterogeneity chi-square tests indicate no significant variation between the selected and unselected samples. Further comparison with the data from unselected tetrads is made possible by calculating the frequency of total proximal events among total single site events (in the three-point crosses, single site events include events which co-convert the temperature sensitive allele with either the proximal allele or the distal allele). Reciprocal events were also included in the total. The use of these data for comparison to the data derived from selected tetrad dissections is valid if parity of single site gene conversion exists. An analysis of the proximal and distal allele conversion described in Table 7 does not indicate any significant departure from parity. Accordingly, the frequencies of proximal allele conversion calculated from the unselected sample were compared to the frequencies of proximal allele conversion calculated from the selected samples. No evidence of discrepancy was found.

Analysis of the proximal allele conversion frequencies shown in Table 13 indicate that the frequency of proximal allele conversion among prototrophic spores is reduced in LZ10 (*30* x *1*). A similar reduction was noted in the analysis of the data from unselected tetrad dissections (Table 7). The relative frequency of conversion of *his1-315* in diploid LZ11 (*315* x *30.1*) is also significantly reduced compared with the conversion frequency of *his1-315* in the other four crosses carrying this marker ($\chi^2_3 = 11.5, p < .05$). This was not seen in the analysis of the data from unselected tetrads (Table 7). This analysis however, used the relative frequency of conversion of *his1-315* to the total events detected

which included co-conversion events.

Flanking Marker Configuration of the Proximal Convertants

As with the unselected tetrads prototrophic spores can be classified on the basis of the flanking marker configuration, as P1, P2, R1 and R2. The specific allele combinations are noted in the previous section, "Reliability of Selection Technique".

Of the 981 proximal convertant spores, 587 are parental for flanking marker configuration. This proportion is not significantly different from that observed in the proximal convertants in unselected tetrads. Tetrads in classes 11-20 of Tables 10 and 12 have the flanking markers of the prototrophic spore in the P1 configuration. These consist of a total of 583 tetrads and can most simply be explained as being due to conversion of the proximal allele. Classes 35 and 36 include prototrophic spores with the P2 parental arrangement of outside markers. These can be the result of exchanges in both the *hom3-his1* region and the *his1-arg6* region or they can represent two strand conversion events. There are a total of four such tetrads.

As expected, the more common recombinant marker combination of flanking markers is R1 configuration. This arrangement is represented in classes 27-34, and includes 268 tetrads. The most probable origin of the prototrophic spores in these tetrads is a proximal conversion event with an exchange taking place either to the proximal or distal end of the conversion event.

Classes 21-26 represent tetrads in which the prototrophic spore has the other recombinant configuration, R2. There is a total of 126 tetrads of this type. These prototrophic spores can be explained most simply by proximal conversion, separated from a distal exchange associated with an "unconverted" segment. It is important to determine whether this

distal crossover in the *his1-arg6* region is a coincidental exchange or is associated with the proximal conversion event as appeared to be in the case of the unselected tetrads. If crossovers in the *his1-arg6* region are occurring at random with respect to conversion of proximal *his1* alleles it is expected that there will be about an equal number of exchanges involving non-conversion chromatids. These exchanges are detected in classes 13, 14, 16, 18, 24, 29, 30 and 36 of Tables 10 and 12. There are only 37 tetrads of this type, whereas 126 would be expected on the hypothesis of independence.

A calculation, analogous to that performed for the unselected tetrads, gives the number of crossovers expected between *his1* and *arg6*, in tetrads of non-R1 configuration as 98 assuming no interference. Half of these (49) should be associated with the chromatid involved in the proximal conversion event. The observations indicate an excessive number of exchange tetrads (163). Since 126 of these exchanges involve the converted chromatid, the excess is attributable exclusively to events involving the converted chromatid. Thus the evidence substantiates the conclusion derived from the unselected data that the distal crossover is associated with the proximal conversion event. Furthermore, the larger sample clears up any doubts as to whether the unconverted strands also have elevated levels of exchanges in this region; they do not.

Distal Allele Conversion

Asci which contained a prototrophic spore, one distal allele and two proximal alleles were judged to be asci which had undergone conversion of the distal allele. In the three-point crosses distal allele conversion may or may not result in co-conversion of the temperature sensitive alleles. All tetrads showing distal allele conversion are described in classes 37 to 53 of Tables 10 and 12.

The relative frequencies of distal conversion among selected tetrads are given in Table 15. A strict comparison of these frequencies in selected and unselected tetrads was not possible due to the small number of prototrophic spores resulting from distal conversion among the unselected tetrads. As an alternative, the frequencies of distal conversion among total single site conversion tetrads (reciprocals included) were calculated for the unselected tetrads and these are included in Table 15. As was stated previously, the validity of this comparison depends on parity of gene conversion. The comparison of distal allele conversion frequencies among selected and unselected tetrads on this basis did not suggest that the frequencies in each sample were different.

The relative frequency of distal conversion among selected tetrads is higher in LZ10 (*30* x *1*) than in the other crosses. This does not necessarily imply that the basic conversion frequency per total tetrads of *his1-1* is increased in this cross. The relative increase seen is probably the concommitant result of the decrease in conversion of *his1-30* which has already been described.

Flanking Marker Configuration of Distal Allele Convertants

Of the 168 prototrophic spores classified as distal convertants, 73 are parental for the flanking marker configuration of *hom3* and *arg6*. The proportion of parental flanking marker configuration accompanying distal conversion is not significantly different in the selected and unselected tetrads. However, the ratio of parental to recombinant flanking marker configuration is significantly different than that observed for proximal allele conversion ($\chi^2 = 15.75, p < .01$).

Of the 73 distal convertants with parental configurations of flanking markers 70 prototrophic spores can be classified as P2 (Tables 10 and 12, classes 37-40) and are assumed to represent spores which have

TABLE 15. Frequency of distal allele conversion in selected and unselected tetrads.

Diploid Strain	SELECTED TETRADS conversion to + total recombinants	UNSELECTED TETRADS conversion to + or m total recombinants*
Z2367 *(315)x(1)*	$\frac{35}{258} = .14$	$\frac{5}{40} = .125$
LZ4 *(315)x(1s)*	$\frac{23}{247} = .09$	$\frac{3}{26} = .12$
LZ10 *(30)x(1)*	$\frac{36}{146} = .25$	$\frac{2}{9} = .22$
LZ6 *(315 1s)x(1)*	$\frac{26}{223} = .12$	$\frac{5}{32} = .16$
LZ11 *(315)x(30 1)*	$\frac{34}{234} = .15$	$\frac{5}{19} = .26$
LZ13 *(315)x(1)*	$\frac{14}{165} = .08$	

* "total recombinants" does not include co-conversion events which encompassed all the markers in the gene

undergone a distal conversion event. There are three tetrads in which the prototrophic spores have the alternative parental array of flanking markers, P1, (Tables 10 and 12, classes 52-53). These can be the result of two crossovers (exchanges in the *hom3-his1* and *his1-arg6* regions) or a two strand conversion event.

Seventy-eight prototrophic spores have the R1 configuration of flanking markers (Table 10 and 12, classes 42-47). These are assumed to be the result of a distal conversion event immediately associated with a distal or proximal exchange.

There are nine tetrads in which the prototrophic spores have the R2 array of flanking markers (Tables 10 and 12, classes 48-51). These tetrads are explained most simply as the result of an exchange in the *hom3* to *his1* region separated from the distal conversion by a segment which does not show conversion. If exchanges in the *hom3* to *his1* region occur at random with respect to conversion of distal *his1* alleles it would be expected that an additional nine tetrads should show an exchange in this region not involving the converted chromatid. The data show only two tetrads of this type (Tables 10-12, class 40). Moreover the total number of tetrads with an exchange in the *hom3* to *his1* can be calculated. Thus among non-R1 tetrads (81), assuming no interference, and using the map distance of 2.5 from Table 2 the number of exchanges expected is 4. The observation of 11 tetrads with an exchange in the *hom3* to *his1* region and the fact that nine are associated with the converted chromatid suggests that these events are not independent, a conclusion similar to that reached in previous consideration of R2 configurations.

Reciprocal Recombination

Asci which contained a prototrophic spore, a double mutant spore, one

distal allele and one proximal allele were classified as reciprocal recombinants. These tetrads are described in classes 1-11 of Tables 10 and 12. The flanking marker configuration of the prototrophs in 82 of these tetrads is R1 (Tables 10 and 12, classes 1-6). This can be the result of an exchange occurring between the proximal and distal alleles. There are seven tetrads with the P1 configuration (Tables 10 and 12, classes 7-10). These could arise from two crossovers, one in the *hom3-his1* region and one between the proximal and distal alleles of the *his1* locus, or from a two strand conversion event.

In four of the diploids the reciprocals are detected between *his1-315* and *his1-1*. The relative frequencies of reciprocal recombinants for each of these diploids is as follows:

for Z2367 (*315* x *1*), the frequency is 0.062;

LZ13 (*315* x *1*), the frequency is 0.073;

LZ6 (*315.1s* x *1*), the frequency is 0.018;

LZ11 (*315* x *30.1*), the frequency is 0.102.

These relative frequencies of reciprocal recombination are significantly heterogeneous ($\chi^2_3 = 14.28$ $p < .01$). This heterogeneity is caused mainly by the decrease shown by diploid LZ6 and cannot be attributed to a distance dependent parameter since in these diploids the event is being detected within the same interval.

The other two diploids used in this study carried markers which are closer together than *his1-315* and *his1-1*. The frequency of reciprocals in LZ4 (*315* x *1s*) is 0.065 and in LZ10 (*30* x *1*) is 0.116. It seems that even using closer markers does not effect an alteration in the frequency of reciprocal recombination as large as the change found in LZ6.

Of the four tetrads from LZ6 which showed reciprocal recombination,

three of the prototrophic spores are temperature sensitive and one is not. None of the markers involved in the three tetrads containing temperature sensitive prototrophs shows an aberrant segregation ratio. The simplest explanation for these tetrads is an exchange between *his1-315* and *his1-1s* ($\frac{315}{+} \times \frac{1s\ +}{+\ 1}$).

The other tetrad, which contains a non-temperature sensitive spore, has a 3:1 (+:m) segregation of *his1-1s*. This tetrad would be explained by a conversion of *his1-1s* accompanied by a crossover either distal or proximal to *his1-1s* ($\frac{315}{+} \times \frac{(1s)\ +}{+\ 1}$ or $\frac{315\ (1s)}{+\ +} \times \frac{+}{1}$).

Twenty-four tetrads from LZ11 were classified as reciprocal recombinants. Seven of these contain temperature sensitive prototrophs and do not show aberrant segregation patterns for any of the markers. These tetrads probably represent the result of an exchange between *his1-30* and *his1-1* ($\frac{315\ +}{+\ 30} \times \frac{+}{1}$). Eleven tetrads contain non-temperature sensitive prototrophic spores and show no aberrant segregation patterns for any of the markers involved. It is suggested that they represent the result of an exchange between *his1-315* and *his1-30* ($\frac{315}{+} \times \frac{+\ +}{30\ 1}$).

The remaining six tetrads contain prototrophic spores which are non-temperature sensitive and show aberrant segregation for *his1-30* (3:1,+:m). These tetrads could result from the conversion of *his1-30* to wild type accompanied by an exchange either distal or proximal to *his1-30* ($\frac{315\ +}{+\ (30)} \times \frac{+}{1}$ or $\frac{315}{+} \times \frac{+\ +}{(30)\ 1}$).

DISCUSSION

The results described in the preceding section indicate that different markers have different conversion frequencies and that marker effect interactions can occur. In particular the analysis of the marker effect of *his1-30* in the three-point cross (LZ11) indicates that the presence of a marker with a low basic conversion frequency and a marker with a relatively high basic conversion frequency results in a reciprocal alteration of both frequencies. This type of interaction is similar to the marker interaction demonstrated in *Ascobolus* (Leblon and Rossignol 1973). Models of recombination which most easily account for these effects are hybrid DNA models in which conversion is predominantly the result of repair of heteroduplex DNA. For this reason the Results are discussed in this section in the context of a hybrid DNA model of recombination. The discussion will be concerned with: (1) independent correction on one chromatid, (2) position of the cross-strand exchange, (3) reciprocal recombination, and (4) marker effects of *his1-30* and *his1-1s*. Under the assumption of a hybrid DNA model of recombination a unifying explanation of most of the observations is possible.

Some of the findings described in the Results were not readily interpretable and will not be discussed further. These findings include: (1) chromatid interference in which the observed numbers of two-strand, three-strand and four-strand double exchanges did not fit the expected 1:2:1 relationship, (2) variation in the ratio of parental to recombinant configuration of flanking markers among proximal and distal convertants, and (3) possible inequality of chromatid involvement in conversion events in diploid LZ4 (*315* x *1s*) noted in the unselected tetrads. However, these observations do not indicate that a hybrid DNA model is inappropriate.

Independent Correction on One Chromatid

Independent correction can be thought of as non-overlapping correction of two sites of mismatches in one length of hybrid DNA. Evidence of independent correction on one chromatid is available in *Neurospora crassa*. Stadler and Kariya (1969) detected an appreciable number of conversion events in three-point crosses in which the two outside sites were converted in the same direction and the middle site was not converted. Similar studies in yeast in which this type of event could be detected indicate that such events are very rare and the opinion of some authors (Fogel *et al.* 1979) is that independent correction generally does not occur in *Saccharomyces cerevisiae*. The argument which Fogel *et al.* (1979) use to dismiss the notion of independent correction is the following: If independent correction were the normal mode of correction along a length of hybrid DNA, then single site events should always be at least as frequent as co-conversion events and their relative frequencies should be independent of the distance between them. However, as their data and the data from this study show, there is an excess of co-conversion events for alleles in close proximity. This they conclude is incompatible with independent correction.

This argument makes the assumption that correction is either independent or not, and by showing that in some instances it is not, they rule out independence as the sole mechanism. However, the possibility remains that correction of nearby sites might overlap while allowing more distant markers to be corrected independently of one another. Furthermore, this correction could be in either direction. The following discussion argues that this form of independent correction does occur in yeast.

The frequent observation of co-conversion of two or more sites within a gene in a co-ordinated manner supports the assumption that a continuous length of hybrid DNA can span more than one site. Correction can also be shown to be continuous. From the data on LZ11 (*315 x 30.1*) it can be seen that *his1-30* has an influence on the correction of *his1-315* and vice versa. This is consistent with the notion that the same correction event can extend to two sites. The data of Leblon (1972), which was discussed in the Introduction, also supports this view.

Although it seems that correction does involve a continuous length of DNA, it can also be shown that it does not necessarily involve the total length of hybrid DNA. Fogel *et al.* (1979) have tetrad data on the *arg4* locus of *Saccharomyces cerevisiae* which show events in which one marker is converted while the adjacent marker shows post-meiotic segregation. These results imply that the hybrid DNA covered both markers but only one marker underwent correction. Thus the possibility for independent correction in yeast exists. Certain tetrads described in this study suggest that independent correction does indeed occur. These tetrads exhibit the R2 configuration of flanking markers and were described (in Results) as containing a spore that most probably resulted from an exchange separated from a converted marker by an un-converted site. In effect, it would be the result of an exchange associated with a length of hybrid DNA in which the allele immediately adjacent to the exchange was restored to the parental genotype, while the more distant allele was converted in the opposite direction. Because this event was very common, it could not be explained simply as conversion with coincidental exchange in the outside region; rather the two events must be associated. Independent conversion is a corrollary of the most satisfying mechanism to explain the association.

Position of Cross-strand Exchange

Models of recombination proposed to account for observed data showing high association of conversion-associated exchange suggest that in almost all cases, hybrid DNA is flanked on at least one side by an exchange.

If a crossover event is symmetrical it is impossible to establish at which end of the conversion length that exchange is. However, if events are asymmetrical then there are specific predictions dependent on whether one postulates that the exchange is outside the gene or at a variable position within the gene.

The result described for recombination at the *his1* locus may be useful in this context since it appears that most events are asymmetrical. Only five events in 1471 tetrads require the involvement of conversion on two chromatids. Other data on yeast (Fogel *et al.* 1971; Fogel and Mortimer 1974; Esposito 1971) also indicate that most events can easily be explained by an asymmetrical distribution of hybrid DNA.

If polarity is the result of variable length of hybrid DNA extending into the gene from a fixed origin, then the polarity pattern evident at the *his1* locus suggests that most hybrid DNA lengths originate proximally. The question to be asked is whether the exchange is at the origin or termination of the hybrid DNA.

The data from the unselected and selected tetrads are summarized in Table 16. Prototrophic events are classified with respect to whether conversion involved the proximal or distal allele (p vs. q) and the configuration of flanking markers (P1, P2, R1, and R2). In the selected tetrads the classes which are relevant to the question of position of the cross-strand exchange are the R1,p class; R2,p class; R1,q class and R2,q class.

TABLE 16. Summary of recombinant spores at the *his1* locus among selected and unselected tetrads.

	SPORE CLASS					
UNSELECTED		**	P1p	R1p	R2p	P2p
	$+\overline{m_1}+$ -	$+\overline{m_1}^{\times}+$ -	$+\overline{m_1}+$ -	$+^{\times}\overline{m_1}+$ -	$+\overline{m_1}+^{\times}$ -	$+^{\times}\overline{m_1}+^{\times}$ -
Z2367	6	4	7	5	0	0
LZ4	7	9	7	7	0	0
LZ10	1	1	1	0	0	1
LZ6	6(6)	4(4)	10	6(1)	1	0
LZ11	4(4)	1(1)	2(1)	4(2)	2(1)	0
Total	24	19	27	22	3	1
SELECTED						
Z2367			122	59	25	1
LZ4			120	67	21	0
LZ10			47	31	15	0
LZ6			114(5)	51(2)	28(2)	0
LZ11			100(55)	52(22)	23(10)	3
LZ13			80	44	14	1
Total			583	304	126	5
UNSELECTED		**				
	$-\overline{+m_2}+$	$-\overline{+m_2}^{\times}+$	$-\overline{+m_2}+$	$-^{\times}\overline{+m_2}+$	$-\overline{+m_2}^{\times}+$	$-^{\times}\overline{+m_2}^{\times}+$
Z2367	0	4	10	5	7	0
LZ4	1	1	5	1	1	0
LZ10	1	0	4	1	0	0
LZ6	9(9)	8(8)	5	2	2	0
LZ11	5(5)	3(3)	6(4)	1(1)	1	0
Total	16	16	30	10	11	0

$\overline{}$ conversion length

$\times$ exchange between *his1* and outside marker

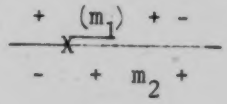
** the exchange was arbitrarily put to the right

Numbers in brackets indicate that the temperature sensitive allele was co-converted with m_1 or m_2 in the three point crosses.

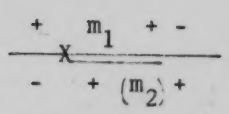
UNSELECTED	SPORE CLASS			
	$+\ m_1^{+}\ -$	$+\ m_1^{\times +}\ -$	$+^{\times} m_1^{+}\ -$	$+^{\times} m_1^{\times +}\ -$
Z2367	1	2	2	0
LZ4	1	0	0	0
LZ10	0	0	0	0
LZ6	0	1(1)	0	0
LZ11	0	4(1)	0	0
Total	2	7	2	0

UNSELECTED	P2q	R1q	R2q	P1q	
	$-\ +m_2\ +$	$-\ +^{\times} m_2\ +$	$-^{\times} +m_2\ +$	$-^{\times} +^{\times} m_2\ +$	Reciprocals
Z2367	0	0	0	0	1
LZ4	1	1	0	0	2
LZ10	1	0	1	0	0
LZ6	1(1)	2(2)	0	1(1)	0
LZ11	0	1	0	0	0
Total	3	4	1	1	3
SELECTED					
Z2367	16	18	0	1	16
LZ4	10	10	2	1	16
LZ10	9	23	3	1	17
LZ6	15(14)	10(6)	1	0	4
LZ11	13(3)	18(4)	3	0	24
LZ13	7	7	0	0	12
Total	70	86	9	3	89

Under the assumption that the exchange takes place outside the gene, then exchanges in the proximal region can give rise to two of the above classes. These are diagrammed as follows:



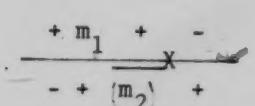
which represents a R1,p class and includes 303 tetrads, and



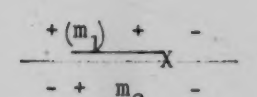
which represents a R2,q class and includes 9 tetrads.

The R1,p class represents an exchange associated with a short conversion length in which m_1 has been corrected to +. The R2,q class represents an exchange associated with a long conversion length in which independent correction has occurred and m_2 has been converted to +.

The remaining two classes cannot be explained by exchanges at the high conversion end and must be the result of exchanges in the distal end. Assuming that the exchange is outside the gene, the configuration of these two classes is as follows:



which represents a R1,q class and includes 86 tetrads, and



which represents a R2,p class and includes 126 tetrads.

The R1,q class represents an exchange associated with a short conversion length in which m_2 has been converted to +, and is analogous to the R1,p class. The R2,p class represents an exchange associated with a long conversion length in which independent correction has occurred and m_1 has been converted to +.

Without further assumptions it would be expected that the relationship of the two kinds of events produced from the proximal exchange would show similarities to the relationship of the two kinds of events pro-

duced by a distal exchange. The data indicate that this is not so, the ratios of the proximal events R1,p:R2,q is 303:9 whereas the ratio of distal exchange events, R1,q:R2,p is 86:126.

Alternatively, if the assumption is that the exchange occurs at a variable position within the gene away from the origin then the exchanges should reflect the polarity seen in the gene such that R1,p would indicate events which ended between m_1 and m_2 and would predominate R1,q and R2,p would indicate events which terminated at the distal end of the gene and should be equal and less common and R2,q will not occur. The observations tend to support this view.

The total selected tetrad data show R1,p = 303, R1,q = 86, R2,p = 126, and R2,q = 9. R2,p is possibly elevated by the inclusion of approximately 37 tetrads which may represent unrelated crossovers in the distal region and similarily R2,q may include 4 unrelated proximal crossovers (see Result section on flanking marker configuration of selected tetrads). The corrected values are R1,p = 303, R1,q = 86, R2,p = 89, and R2,q = 5. The presence of some tetrads in the R2,q category may reflect a very low level of events coming in from the distal origin.

Generally the data give little support to the hypothesis that the exchange is outside the gene and do not differ much from expectations based on the assumption that the exchange is at a variable position within the gene and that most events within the *his1* locus are related to the proximal end of the gene. This supports the configuration used in the Holliday (1964) and Meselson and Radding (1975) models and is in strong disagreement with the configurations postulating exchanges outside the gene i.e., Whitehouse and Hastings (1965).

Fogel *et al.* (1979) have published some data which are relevant to the question of the position of the cross-strand exchange. They have

analyzed tetrads in which one of the markers at the *arg4* locus in yeast shows a high frequency of post-meiotic segregation. The position of the exchange in these tetrads can be determined to be distal or proximal to the heteroduplex DNA without ambiguity. Their finding is that the associated exchange can occur either proximal or distal to the site of post-meiotic segregation.

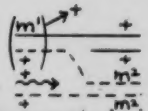
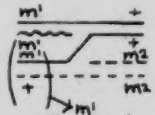
A further confirmation of this finding is available for the *his1* locus. Linda Freidman (unpublished results) has data on a cross carrying *his1-40*. This allele shows high post-meiotic segregation. In seven tetrads showing post-meiotic segregation, three showed post-meiotic segregation associated with a proximal exchange and one showed post-meiotic segregation associated with a distal exchange. These results on post-meiotic segregation are difficult to reconcile with the Meselson-Radding model. However, a further discussion of this point in relationship to the model is presented in the discussion of reciprocal recombination.

Reciprocal Recombination

The tetrads which contain one prototrophic spore, one double mutant spore and one each of the parental alleles were classified as reciprocal recombinant tetrads. The results of the analysis of these tetrads from the selected tetrad dissections are as follows: (1) In all crosses the frequency of reciprocal recombination is lower than the frequency of proximal or distal conversion; (2) Comparison of the frequency of reciprocal recombination in Z2367 (*315* x *1*), LZ4 (*315* x *1s*) and LZ10 (*30* x *1*) indicate it is not entirely distance dependent; (3) Results for LZ11 (*315* x *30.1*) indicate that the exchange is not confined to a specific region; (4) Results from LZ6 (*315.1s* x *1*) and LZ11 (*315* x *30.1*) indicate that conversion may be associated with the reciprocal event; (5) Results

from LZ6 (*315.1s* x *1*) show that an additional site of distal heterozygosity can lower the frequency of reciprocal recombination.

The observations on reciprocal recombination which show that it can be associated with conversion and that the exchange can occur at a variable position within the gene support the assumption that the same kind of general conversion event which gave rise to other recombinant tetrads is responsible for reciprocal recombination.

The R1,p event which gives rise to a prototrophic spore by proximal conversion is assumed to look like this  in which the conversion of m_1 is to +. The equivalent event initiated on the other chromatid would not give rise to a prototroph but would give rise to a double mutant spore. 

The results from unselected tetrads show that the conversion events on two chromatids are in the ratio of 107:92. This suggests that heteroduplex formation occurs equally frequently on both chromatids. Furthermore, in general, proximal allele conversion shows parity. Thus a heteroduplex heterozygous for mutant and wild type is equally likely to be converted to mutant or wild type. It should follow then that the two configurations shown above should be equally likely to be corrected to parental and then give rise to reciprocal recombinant tetrads R1,r.

The data show that this is not occurring at the frequency expected. For example, in cross Z2367 (*315* x *1*), there are 16 R1,r tetrads and 59 R1,p tetrads. It would be expected on the above analysis that R1,r should actually exceed R1,p, that is R1,r should equal 2R1,p. Therefore, it seems that if reciprocal recombination were the result of this essentially conventional conversion mechanism, then the restoration of a heteroduplex back to the parental strand is not occurring at the same

frequency as the frequency of conversion in the other direction. In the example given (Z2367) it is observed that there are 16 R1,r tetrads and 108 were expected. Thus it can be calculated that a heteroduplex, heterozygous for *his1-315*, is eight times more likely to be corrected to the genotype of the invading strand than to that of the invaded strand.

There is evidence to support this idea of strand disparity in other organisms. Nevers and Spatz (1975), using lambda heteroduplex molecules carrying a mutant allele on one strand and its wild type allele on the other strand, showed that conversion showed no preference for one of the two markers but rather for one of the two strands. Regardless of the orientation of the marker, conversion to the r strand genotype occurred almost twice as often as conversion in the opposite direction.

In an earlier study, Spatz and Trautner (1970) showed that this disparity in strand preference was in part allele specific, however, the chemical nature of the mismatched bases was not the only factor involved in the conversion pattern. A model of genetic recombination based on preferential strand correction has also been suggested for eukaryotes. Kushev (1974) proposed a theory of "directed" correction in which the correction preferentially occurs on the donor chromatid. His model proposes a symmetrical distribution of heteroduplex formation and several assumptions to accommodate available data. He suggests also that the degree of directedness of correction can vary with different markers.

The results presented here suggest that conversion restoration disparity may have allele specific parameters. The results of cross LZ10, which show an increase in reciprocal recombination indicate that *his1-30* must have a different disparity coefficient from *his1-315*. It can be calculated to have a 3.6:1 disparity favoring the invading strand, compared with 8:1 for *his1-315*. Detectable conversion of *his1-30* allele

is also reduced.

Although the conversion restoration disparity of proximal alleles can account for variation in reciprocal recombination in LZ10, the reduced amount of reciprocal recombination in LZ6 (*315.1s* x *1*) cannot be accounted in the same way. This cross carries the same proximal marker, *his1-315*, as the other crosses showing higher frequencies of reciprocal events. In this cross, it is as though the extra distal sites are blocking the entry of the cross-strand exchange into the *315-1* region and they must be doing it by preventing the entry from a more distal point. Since most evidence suggests that events are initiated in the proximal region it seems the only satisfactory explanation is that the cross-strand exchange can migrate in either direction. Sigal and Alberts (1972) have suggested that this can occur as a consequence of rotary diffusion of the two DNA molecules. Several scenarios can be devised to account for the observations; the following is one: Polymerase activity might drive the exchange from the proximal origin, but after isomerization, the exchange might then migrate back into the gene toward the origin. If, as is possible the act of correcting mismatches blocks the migration back into the gene, then the additional site of heterozygosity in the distal region could increase the likelihood of a correction complex becoming attached and thereby prevent back migration.

Analysis of the selected tetrads of LZ6 (*315.1s* x *1*) failed to show any other significant differences from the two-point cross L2367 (*315* x *1*). It would be predicted that if migration of a cross-strand exchange back into the gene is reduced, then hybrid DNA length would be increased and this might be then translated into the increased co-conversion seen in the unselected tetrads. This reason for increased co-conversion should result in an increase in frequency of conversion of distal markers. This

is apparent in the results but is not considered significant.

From the preceding argument, that correction of mismatches may prevent the migration of a cross-strand exchange back into the region under study, it should follow that uncorrected mismatches would allow the migration back into the gene. If these uncorrected sites were formed in the asymmetrical phase of a Meselson and Radding model the migration of the cross-strand exchange back past these sites would not have the effect of restoring homoduplex, but would in effect maintain heteroduplex. This site, if left uncorrected, would give rise to post-meiotic segregation with the possibility of an exchange in the proximal region. Thus it would seem possible that if sites showing high post-meiotic segregation are sites which fail to initiate correction they could show an association with either proximal or distal exchange. The results discussed previously in the section on position of the cross-strand exchange indicates that they do.

Marker Effect of *his1-30*

A summary of the data involving the *his1-30* allele is as follows: (1) The two crosses carrying *his1-30* have reduced numbers of detectable recombinational events. The reduction is greater in LZ10 (*30* x *1*) than in LZ11 (*315* x *30.1*) (Table 9); (2) The presence of *his1-30* as a proximal marker in cross LZ10 causes a significant decrease in the conversion frequency of the distal marker, *his1-1* (Table 9); (3) The inclusion of *his1-30* as a mid-marker in cross LZ11 causes a significant reduction in the conversion frequency of the proximal marker, *his1-315* (Table 9); (4) The conversion frequency of *his1-30* is increased in cross LZ11 as compared to its conversion frequency in cross LZ10 (Table 9); (5) The frequency of reciprocal recombination events among selected tetrads appears to be increased in crosses involving *his1-30*.

There are various explanations which can be put forth to explain the behavior of *his1-30* and its effect on recombination at the *his1* locus. In part the reduced frequency of events detected in LZ10 might be due to the position of *his1-30* in the gene and the pattern of polarity of gene conversion. Given that *his1-30* is located toward the middle of the *his1* gene and the pattern of polarity reflects a variable extension of hybrid DNA into the gene, from a proximal origin, it would follow that *his1-30* would be included in heteroduplex relatively infrequently and would therefore show a reduced frequency of events when compared to crosses carrying a more proximal marker. This effect of *his1-30* would be overcome by the presence of a more proximal allele, i.e., *his1-315*. However, LZ11 shows an overall decrease in detectable events in the presence of *his1-315*, so that this explanation is not particularly satisfying. There are several more tenable possibilities.

LZ10 and LZ11 might both possess a genetic background which affects the frequency of recombination. Genes which alter recombination are well known in both prokaryotic and eukaryotic systems (for reviews see Catcheside 1974, 1977). Certain of these genes can be described as genes which have a general effect on recombination. These genes reduce or eliminate recombination throughout the genome. Examples of these include the *recA* mutants in *E. coli*, the *mei1* mutant in *Neurospora*, the *con* mutants in *Saccharomyces* and the *c(3)G* mutant in *Drosophila*. In eukaryotes these mutants are recessive and interfere with normal meiosis. The result of this may be detected by increased sterility, increased nondisjunction and reduced recombination. It is unlikely that a gene of general effect is being expressed in LZ10 or LZ11. Recombination is normal in other parts of the genome and there was no evidence of decreased sporulation or inviability of the meiotic products. Thus if the reduction

in recombination is due to the genetic background of LZ10 and LZ11, it must be due to a gene which has a much more local effect, i.e., recombination at *his1*.

Several genes with this type of local effect are well known in *Neurospora crassa*. The dominant variants of these genes (*rec1*$^+$, *rec2*$^+$, and *rec3*$^+$) act as repressors of allelic recombination at other, specific loci and may also reduce crossing-over in the interval adjacent to the target loci. *Rec1*$^+$ causes a reduction of allelic recombination at *his1* but does not appear to affect non-allelic recombination (Thomas and Catcheside 1969). It also reduces allelic recombination at the *nit2* locus and affects crossing-over around it (Catcheside 1970, 1974). *Rec2*$^+$ is a dominant repressor of allelic recombination at the *his3* locus and of crossing-over between *his3* and *ade3* (Angel *et al.* 1970). *Rec3*$^+$ reduces allelic recombination at the *am1* locus (Catcheside 1966) and also reduces allelic recombination at the *his2* locus and intergenic recombination between *sn* and *his2* (Catcheside and Corcoran 1973). Typically the action of the *rec*$^+$ allele affects a reduction or even a reversal of polarity of conversion at the target locus (Catcheside 1977) and this is reflected by changes in the P1:P2 ratios of the recombinant spores. The suggestion put forth to account for this observation was that the *rec*$^+$ allele reduces the recombination entering from the high conversion end and allows for residual recombination from the opposite end.

The observations on LZ10 and LZ11 are not strictly analogous with those described for the *rec* genes. For example, crosses carrying *his1-30* give no indication that recombination from *hom3* to *his1* or from *his1* to *arg6* is altered in any way. With respect to the configuration of flanking markers there is no indication that events are being preferentially excluded from one end or the other. The P1:P2 ratio in LZ10 is 48:9

and in LZ11 is 102:16. These ratios are not significantly different from that of the standard cross Z2367 (126:17). Furthermore, although there is significant reduction in intragenic recombination events in both crosses it is significantly greater in LZ10 than in LZ11 (Table 9).

An example of another genetic variant which affects recombination in *Neurospora* was discovered in the *rec2-his3* system (Angel *et al.* 1970). This particular gene is called *cog* and was discussed briefly in the Introduction. Its variants are expressed only in the absence of $rec2^+$. In *rec2* crosses the frequency of allelic recombination in *his3* is about six times as great in cog^+ crosses as in *cog* x *cog* crosses. *cog* is linked to *his3* and cog^+ is completely dominant, for no difference in frequency is apparent between cog^+ x *cog* and cog^+ x cog^+ crosses. Differences are apparent however in different cog^+ x *cog* crosses. In cog^+ heterozygous crosses the *his3* site on the cog^+ chromosome undergoes preferential conversion. It has been suggested that heteroduplex occurs preferentially on the cog^+ chromatid and that perhaps as a rule heteroduplex occurs on only one chromatid in a tetrad.

There is good evidence to suggest that the *cog*-like situation does not exist in the crosses carrying *his1-30*. Complete analogy would require that the three diploids with high recombination be cog^+. Since Z2367 supplied the chromatid carrying the markers - *his1-1* ++ for LZ10 and the + *his1-315* -- chromatid for LZ11, it would be expected that if a cog^+-like gene was linked to the *his1* locus, then one or both of these crosses should be carrying the dominant allele and show normal levels of detectable events, and show preferential conversion on one strand.

Although the data from the crosses carrying *his1-30* do not show a strict analogy to the results in *Neurospora crassa* there does remain a possibility that there is a modifier of recombination present in these

crosses. This possibility could be tested genetically. It should be noted that to date, no modifier of recombination of this type has been isolated in *Saccharomyces cerevisiae*.

The remaining possible explanations of the reduction of recombinational events detected in crosses carrying *his1-30* attribute the effect directly to the nature of the allele itself. It could be that the heteroduplex formed at this site results in a specific mismatch which is preferentially restored to parental genotype. This model should produce evidence of strand disparity, since the mismatch would not be the same in the alternative heteroduplex; however, detectable heteroduplex formation on each chromatid was equal in each cross. It is possible to postulate that preferential restoration of the mismatch to the parental strand occurs regardless of which strand the heteroduplex is on. For example, initiation of correction preferentially on the invading strand would produce such an effect and reduce detection of recombination events. This would not necessarily interfere significantly with polarity providing it entailed co-restoration of distal markers to the parental form as well. This latter feature would result in decreased conversion of distal markers (as was observed in LZ10). This explanation also accounts for the results with LZ11. The decrease in conversion frequency of *his1-315* in the three point cross would be the result of initiation of correction at *his1-30*, with its restoration and subsequent co-restoration of *his1-315*. The increase in conversion frequency of *his1-30* in the three point cross could reflect correction initiated at *his1-315*, where there is no reason to suppose the same strand disparity of correction as at *his1-30*. This conclusion that different alleles could exhibit different degrees of conversion restoration disparity was also reached by the analysis of reciprocal recombination discussed in the previous section.

In view of the preceding discussion it seems reasonable to suggest that the marker effect of *his1-30* is due to a preferential initiation of correction of heterozygosity at that site on the non-parental strand, i.e., the invading strand. A molecular basis for this is not suggested, however the site may have some unusual properties with respect to repair. As was noted in the Materials and Methods, a characteristic of this mutant is a high induced homoallelic reversion rate.

Marker Effects of *his1-1s*

The results of Z2367 and LZ6 were compared to see if the inclusion of *his1-1s* as an additional site of heterozygosity caused a change in the conversion spectrum of *his1-315* and *his1-1*.

The data from the unselected tetrads showed that the frequency of detectable recombination events was the same for the two crosses, thus *his1-1s* was not causing a gross disturbance in recombination such as that caused by the presence of *his1-30*. The significant effect detected in the unselected tetrads was an increase in co-conversion of *his1-315* and *his1-1* in the three-point cross when compared to the two-point cross. The frequency of conversion of *his1-315* was consistent for the two crosses ($\chi^2 = .14$, $p = .7$). However, the frequency of conversion of *his1-1* appeared to be increased. The increase was not shown to be significant although the χ^2 approaches significance ($\chi^2 = 1.8$, $p = .18$). However, if the increase in co-conversion of *his1-315* and *his1-1* were entirely at the expense of single-site conversion it could be argued that co-conversion should have an impact by reducing single-site conversion of the allele with the lower frequency of conversion, i.e., *his1-1*. Then, in the selected tetrads, which detect only single site converion but for which data is more substantial, a decrease in distal convertants

should be apparent. The amount of distal conversion among selected tetrads in the two crosses cannot be shown to be different ($\chi^2 = .39$, $p = .5$).

The one significant difference which was detected between the selected tetrads from the two crosses is with respect to reciprocal recombination, as was discussed in a previous section (Reciprocal Recombination). The explanation offered for the observed decrease in reciprocal recombination was that the extra site of distal heterozygosity resulted in an increased probability of correction and subsequently prevented the migration of a cross-strand back into the gene. This resulted in an apparent increase in the extent of hybrid DNA due to stabilization of the half-chromatid chiasma in a more distal position. This explanation is also consistent with the data from unselected tetrads, which show increased co-conversion and probable increase in distal conversion. Thus, the marker effect shown by *his1-1s* may not be an allele specific effect, but may be attributable to the extra site of mismatch which allows for greater probability of distal correction.

Summary

The results of the analysis of conversion in five related diploids indicates an overall consistency in conversion patterns. The recombination pattern in all crosses indicates an asymmetrical distribution of hybrid DNA formation, a strong proximal polarization of conversion frequencies and an excess of distal exchanges. The pattern that emerges is consistent with the formation and distribution of hybrid DNA predicted by a Meselson and Radding model of recombination (1975).

Reciprocal recombination is thought to occur by the same conversion mechanism which gives rise to proximal convertants with an associated exchange. The two different outcomes of the event depend on the direction of correction of the heteroduplex. The relative rarity of reciprocal recombination is accounted for by postulating a strand disparity in the correction of the heteroduplex. The results suggest that heteroduplex, which consists of an invading strand and an invaded strand, undergoes correction on the invaded strand more often than on the invading strand.

Independent support of this notion comes from the marker effect shown by *his1-30*. This allele has a low basic conversion frequency and imposes this on alleles near it. Event frequencies in crosses involving *his1-30* are also decreased. The observations are most easily explained by suggesting that the site of *his1-30* shows more correction on the invading strand than did the other markers. The expectation of this, in light of the previous discussion of reciprocal recombination, would be that crosses carrying *his1-30* would show higher levels of reciprocal recombination. The crosses carrying this allele did show relatively higher frequencies of reciprocal events. *His1-30* is one of the very few markers in yeast which has been shown to have an appreciable marker

effect on other alleles in a cross. The other example was the deletion described by Fink (1974).

The inclusion of *his1-1s* in the three-point cross did not show the dramatic marker effect of *his1-30*. However, it is apparent that the extra site of heterozygosity does have an effect. Co-conversion is increased and this increase is not totally at the expense of single-site conversion. Evidence indicates that the frequency of conversion of *his1-1* is increased. The second observation of an effect of *his1-1s* is a reduction of reciprocal recombination. These two observations are compatible with each other if one supposes that once isomerization occurs in a Meselson and Radding model of recombination the cross strand is free to travel in both directions. It may be that movement towards the proximal end of the gene is prevented if correction has occurred. The presence of two distal sites may increase the probability of correction and thereby prevent a proportion of the exchanges from re-entering the region under study. At the same time this allows for an increase in co-conversion and should result in an increase in the conversion frequencies of the distal alleles. The results suggest that this is probably so.

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